Indonesian J. Pharm. Vol. 28 No. 4 : 191 – 197
ISSN-p : 2338-9427
DOI: 10.14499/indonesianjpharm28iss4pp191

Research Article

THE EMPLOYMENT OF UV-SPECTROSCOPY COMBINED WITH MULTIVARIATE CALIBRATION FOR ANALYSIS OF PARACETAMOL, PROPYPHENAZONE AND CAFFEINE

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Submitted: 08-10-2017
Revised: 15-11-2017
Accepted: 20-12-2017

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ABSTRACT

The reference method for simultaneous analysis of drugs is chromatography, however, this technique is expensive, complex, and needs excessive sample preparation; therefore, some simple methods like UV spectroscopy is proposed. Assisted with multivariate calibration, it is possible to analyze drugs using UV spectroscopy without prior separation. This study is intended to use UV spectroscopy coupled with multivariate calibration of partial least square (PLS) for simultaneous analysis of paracetamol (PCT), propyphenazone (PROPI), and caffeine (CAFF) in tablet dosage form. The calibration model was prepared by developing a series 20 mixture of PCT, PROPI and CAFF with certain composition randomly and its absorbance was measured at wavelength of 220-313nm with an interval of 3nm. The performance of calibration model was assessed by coefficient of determination (R^2), root mean square error of calibration (RMSEC) and root mean square error of cross validation (RMSECV). The R^2 values for the correlation between actual values of PCT, PROPI and CAFF and predicted values using UV-spectroscopy combined with PLS were 0.9994; 0.9878; and 0.9919, respectively. The calibration errors expressed with RMSEC were 0.027%, 0.082% and 0.043% for PCT, PROPI and CAFF, respectively. While, during cross validation using "leave one out" technique, RMSECV values obtained were 0.062%, 0.095% and 0.982%, respectively for PCT, PROPI and CAFF. The level of drugs obtained were 226.76±14.49 mg/tablet (equivalent to 90.70% from labeled claim) for PCT, 135.74±11.23 mg/tablet (equivalent to 90.49% from labeled claim) for PROPI and 51.69±2.35 mg/tablet (equivalent to 103.38% from labeled claim) for CAFF.

Keywords: UV-spectrophotometry, multivariate calibration, partial least square, paracetamol, caffeine, and Propyphenazone.

INTRODUCTION

The drug formulations containing three active components are intended to enhance the pharmaceutical effects of each substance and to cover a larger medical treatment. Pharmaceutical dosage form containing paracetamol (PCT), propyphenazone (PROPI) and caffeine (CAFF) are used as analgesic and antipyretic which have been reported to have synergetic effect. These mixture are more effective than paracetamol, ibuprophen, and aspirin given individually (Kiersch and Minic, 2002; Soponar *et al.*, 2013). The chemical structures of PCT, PROPI and CAFF (Figure 1).

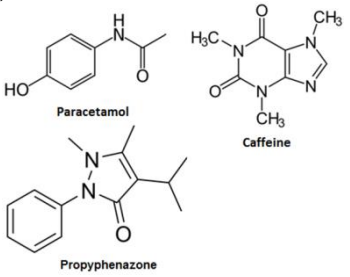

Figure 1. The chemical structures of paracetamol, propyphenazone and caffeine

Analysis of drugs in the mixture is performed using chromatographic techniques due to the capability of chromatography to separate and to quantify the analyte(s) of interest. HPLC using diode-array detector has been used for analysis PCT, PROPI and CAFF in tablet (Soponar *et al*., 2013). Micellar electrokinetic capillary chromatography (Emre and Ozaltin, 2007), thin layer chromatography (Dimitrovskat *et al*., 1995), and gas chromategraphy (Markoviz and Kusez, 1992) have also used for analysis of PCT, PROPI and CAFF in the pharmaceuticals. However, chromatographic and electrophoretic techniques involve time consuming, skillful analyst, organic reagents and involving excessive sample preparation. Therefore, a simple and rapid technique is developed for analysis of drugs in the complex mixture without separation step such as UV spectroscopy. The use of UV spectroscopy for analysis of complex mixture is possible with the aid of several Chemometrics techniques (Rohman, 2012).

Spectrophotometry, either normal or derivative spectra, combined with some calibration techniques such as partial least square (PLS) has been used for analysis of PCT, PROPI and CAFF in two or more combinations, some with other drugs (Dinc and Onur, 1995; Dinc, 1999; Bozdogan *et al*., 1992). A multiparameter-responding flow-through system with solid phase UV spectrophotometric detection (a multiparameter optosensor) has also been used for analysis of PCT, PROPI and CAFF in pharmaceuticals (Domínguez Vidal *et al*., 2003). However, analysis of these three drugs using UV spectrophotometry combined with PLS has not been reported yet. Therefore, in this study, the spectroscopy UV in combination with PLS is employed for quantitative analysis of PCT, PROPI and CAFF in tablet dosage forms.

Partial least square (PLS) calibration is one of the inverse calibration (Rohman, 2012), and is calculated using least squares algorithms. PLS is intended to establish a linear correlation between two matrices, the spectral data X and the reference or actual values of Y. In PLS, the matrices X and Y are modeled in order to find out the variables in X matrix capable of describing the Y matrix (Wold *et al*., 2001). PLS is the calibration technique widely used in analysis of multicomponent in pharmaceuticals as reviewed by Roggo *et al*. (2007).

MATERIALS AND METHODS

Paracetamol (PCT) was obtained from PT. Combiphar (Jakarta, Indonesia), while propyphenazone (PROPI) and caffeine (CAFF) were kindly given by PT. Konimex (Surakarta, Indonesia). The tablet used is labeled to contain 250 PCT, 150mg PROPI, and 50mg CAFF and purchased from pharmacy in Yogyakarta, Indonesia. The chemicals and reagents used were of pro analytical grade.

Instrumentation and Software

Spectrophotometer UV-Vis (Shimadzu, Japan) type UV 1800 equipped with quartz cuvette 1 cm (Hellma, USA) was used for UV spectra acquisition. The UV spectral data were exported to Excel (Microsoft Inc., USA) and manipulated using Minitab software version 16 (Minitab Corp., USA). All measurements were performed using 1cm quartz cells over the wavelength range of 200-400 nm with 3nm interval.

Preparation of calibration standards

The standard solutions were prepared freshly. In three separate volumetric flasks 100mL, an approximately of 50mg of each drugs (PCT, PROPI and CAFF) is accurately weighed using semi micro balance (sensitivity 0.01mg), dissolved in 5mL ethanol and made to volume with distilled water to obtain stock solution 50mg drug/100mL. The stock solution is used for preparing calibration samples (20 samples) composed randomly using Excel (Microsoft Inc., USA), (Table I). Some synthetic mixtures with certain concentrations were also prepared to be used in validation samples (10 samples). Each solution mixture was scanned using UV spectrophotometer at 200-400nm. Each 3nm, their absorbance were recorded and used for the optimization of calibration models.

Analysis of PCT, PROPI and CAFF in tablet dosage forms using UV spectrophotometry

Twenty tablets were taken and subjected to weighting for homogeneity test. The tablets are crushed until homogenous. In volumetric flask 100mL, an amount of powder equivalent

to one tablet is taken and dissolved with 10mL ethanol and made to volume. The solution is shaken vigorously for 30min, filtered using Whatman paper, and the supernatant is taken and subjected to spectrophotometric measurement as described above. The concentration of PCT, PROPI and CAFF in tablet dosage forms is calculated based on the optimized calibration model. All determinations were performed in six times.

Data analysis

The levels of PCT, PROPI and CAFF in tablet dosage is calculated with the aid of multivariate calibration of Partial Least Square (PLS). The PLS analysis is carried out using Minitab software version 16 (Minitab Corp., USA). The correlation between actual values of these drugs and predicted values using UV spectrophotometry in combination with PLS is performed using Excel (Microsoft Inc., USA).

RESULTS AND DISCUSSION

Figure 2 revealed the overlay of UV spectra of paracetamol (PCT), propyphenazone (PROPI) and caffeine (CAFF) scanned at UV region (200-400nm). These spectra showed an extensive overlapping which limited the determination of these drugs using UV spectroscopy, simultaneously. Fortunately, some chemometrics techniques like multivariate calibration of partial least square (PLS) are developed to overcome this problem (Mohamed and Mikre, 2009; Rohman *et al.*, 2015). Analysis of drugs in multicomponent system aided with multivariate calibration is done in three separate steps, namely calibration, validation, and analysis of unknown samples.

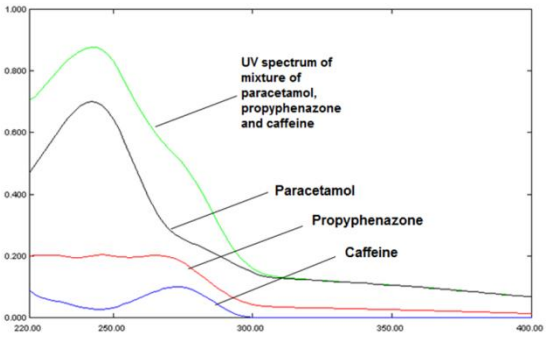

Figure 2. The UV spectra of paracetamol, caffeine, and propyphenazone as well as the mixture of three drugs, scanned at wavelength of 200-400 nm.

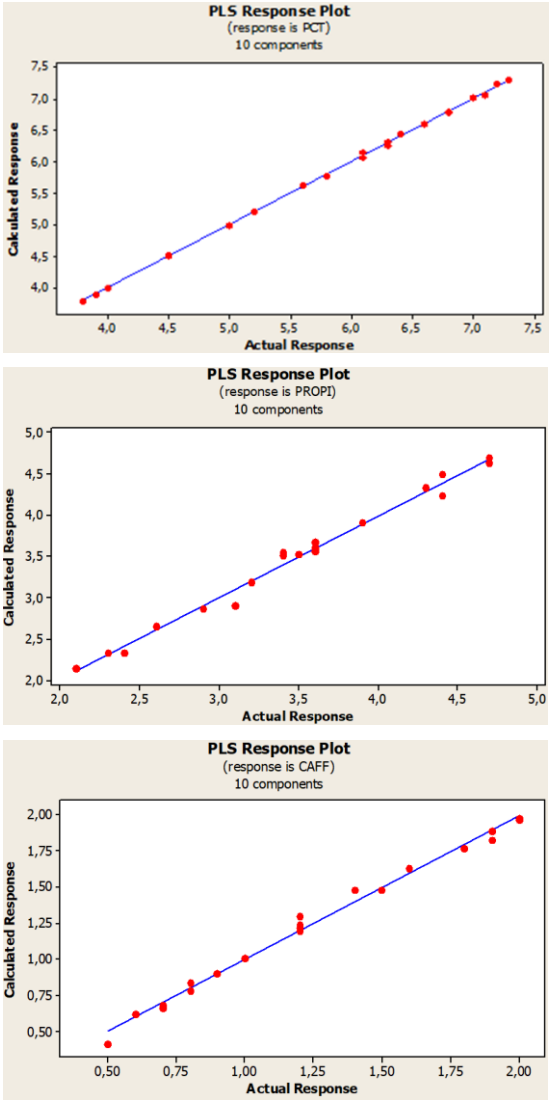

Figure 3. The PLS calibration model for the relationship between actual values of paracetamol (above), propyphenazon (middle), caffeine (below) and UV-spectroscopy predicted values.

The first step is to optimize the wavelength capable of providing the best calibration model, which offer the good correlation between actual values of PCT, PROPI and CAFF and predicted values using UV spectroscopy-PLS expressed by high value of coefficient of determination (R^2). The R^2 value can be used as validation for parameter of accuracy, because R^2 describes the closeness of

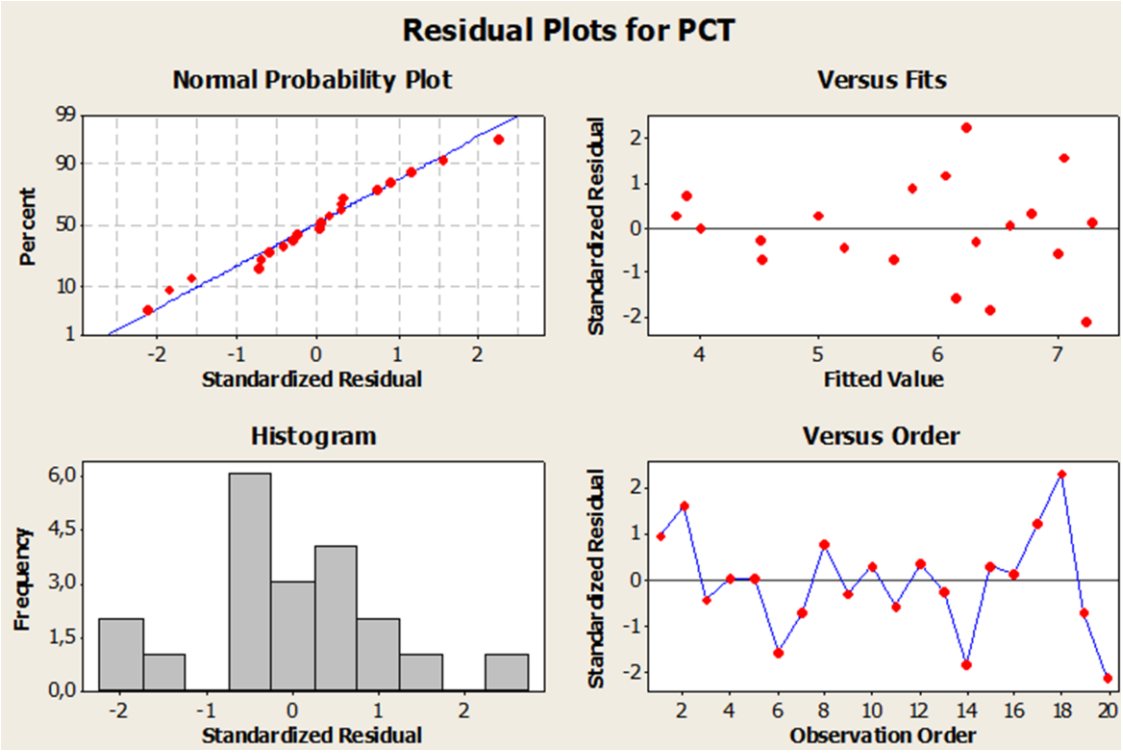

Figure 4. The residual plot of paracetamol describing the difference between actual values and predicted values of paracetamol

the predicted value with the actual value. In addition, the wavelength used during analysis is selected based on its capability to give the lowest errors, either in calibration or validation, expressed with root mean square error of calibration (RMSEC) and root mean square error of cross validation (RMSECV). RMSEC and RMSECV can be used as precision parameters, because these values describes the degree of agreement among predicted values results, when the procedure is applied repeatedly to multiple calibration samples. Based on that criteria, the wavelength of 220-313nm was finally chosen for quantitative analysis of these three drugs (PCT, PROPI and CAFF) simultaneously due to its capability provide the highest values of R^2 and the lowest values of RMSEC and RMSECV.

The equation used for the correlation between actual values (x-axis) and predicted values (y-axis) along with R^2, RMSEC, and

RMSECV values is compiled (Table II). Figure 3 revealed the calibration model of PCT, PROPI and CAFF using PLS regression. In addition, to figure out the random errors occurring during the calibration model, an example of the residual profile of PAR was provided (Figure 4). It is clear that random error occurred, while systematic error is negligible because the residual point existed above and below of target value (zero difference between actual and predicted value).

The calibration model is further subjected to validation using external validation technique. During external calibration, a set samples (10 samples) used as validation samples. The acceptance criteria of external calibration was based on R^2 for correlation between actual value and predicted values in validation samples and RMSECV values. The high value of R^2 and low values of RMSECV (Table III) indicated that the calibration model is suitable for determination of PCT, PROPI and CAFF in unknown samples provided that the samples contain the same drugs in the calibration and validation drug samples.

Table I. Composition of synthetic mixture consisting of Paracetamol (PCT), Propyphenazone (PROPI), and caffeine (CAF) in calibration standard.

No	PCT (µg/mL)	PROPI (µg/mL)	CAFF (µg/mL)
1	5.8	3.5	1.5
2	7.1	3.1	1.2
3	5.2	3.4	0.5
4	6.6	4.7	0.6
5	4.0	4.3	0.7
6	6.1	3.6	0.8
7	5.6	4.4	2.0
8	3.9	3.9	0.9
9	6.3	2.1	1.8
10	3.8	2.3	1.0
11	7.0	3.6	2.0
12	6.8	2.9	1.9
13	4.5	4.4	1.4
14	6.4	2.6	0.7
15	5.0	3.6	1.2
16	7.3	16	1.6
17	6.1	2.4	0.8
18	6.3	4.7	1.2
19	4.5	3.6	1.2
20	7.2	3.4	1.9

Table II The equation, coefficient of determination (R^2) and root mean square of calibration (RMSEC) for paracetamol (PCT), popiphenazone (PROPI) and caffeine (CAFF).

	PCT	PROPI	CAFF
Equation	y = 0.9994x + 0.0034	y = 0.9878x + 0.0426	y = 0.9919x + 0.0101
R^2	0.9994	0.9878	0.9919
RMSEC	0.027445698	0.082897315	0.043408954

Table III The equation, coefficient of determination (R^2), root mean square error of cross validation (RMSECV) and predicted residual error sum squares (PRESS) for paracetamol (PCT), popiphenazone (PROPI) and caffeine (CAFF).

	PAR	PROPI	CAFF
Equation	y = 1.0125x - 0.071	y = 0.9919x + 0.0358	y = 0.9718x + 0.035
R^2	0.993	0.931	0.940
RMSECV	0.0620	0.0954	0.0630
PRESS	0.156	0.782	0.279

The final step of analysis using PLS calibration model is the evaluation of over-fitting using cross validation technique. Overfitting of multivariate calibration is understood that the calibration model can generate good model in the calibration data sets, but it fails to provide optimistic model in the validation samples (Miller and Miller, 2005). During cross-validation with "leave-one out" technique, one of the calibration samples is removed from PLS model, and the remaining samples are used to make PLS model.

Furthermore, the left out 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 samples is computed and is expressed as root mean square error of cross validation. The sum of the squares of these differences is known as PRESS (predicted residual error sum of squares). The smaller the RMSECV and PRESS values, the better the predictive power of the calibration model (Rohman and Man, 2011). Table III also compiled the parameter values of external validation for PCT, PROPI and CAFF using PLS models.

The developed method was further used for determination of tablet formulation containing PCT, PROPI and CAFF. The level of drugs obtained are 226.76±14.49 mg/tablet (equivalent to 90.70% from labeled claim) for PCT, 135.74±11.23 mg/tablet (equivalent to 90.49% from labeled claim) for PROPI, and 51.69±2.35 mg/tablet (equivalent to 103.38% from labeled claim) for CAFF. This result confirms that UV spectroscopy in combination with multivariate calibration of PLS is an alternative method compared to reference method (high performance liquid chromatography) for simultaneous analysis of PCT, PROPI and CAFF in tablets containing these three components.

CONCLUSION

Based on the high value of R^2 as well as low values of RMSEC and RMSECV, it can be concluded that UV spectroscopy coupled with multivariate calibration of PLS is an alternative techniques for simultaneous determination of PCT, PROPI and CAFF in tablet containing these drugs without any separation procedure. The method is rapid and promising for fixed formula. However, to be used in another formulation, this model should be redeveloped

ACKNOWLEDGEMENT

The authors thank to Faculty of Pharmacy for financial support during this study. We also thank to PT. Combiphar (Jakarta, Indonesia) for giving us paracetamol and PT. Konimex (Surakarta, Indonesia) for providing propyphenazone and caffeine standards.

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