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Research Article

Chemometrics-Assisted UV Spectrophotometric Method for Simultaneous Determination of Paracetamol and Tramadol in Divided Powder Dosage Form

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ABSTRACT
Paracetamol and tramadol were commonly prescribed in combination as an anti-inflammatory agent. In Indonesia, a combination of these two drugs was compounded as divided powder dosage form. It was important to ensure the content uniformity of each compound to implement the patient-oriented medication. UV spectrophotometric combined with chemometrics techniques were developed to quantitatively analyze the content of paracetamol and tramadol in divided powder dosage form. Two multivariate calibration method namely principal component regression (PCR) and partial least squares (PLS) were applied in this study. After considering several statistical parameters such as coefficient of determination (R^2), root mean square error of calibration (RMSEC), root mean square error of cross-validation (RMSECV), and root mean square error of prediction (RMSEP), the PLS model was chosen to be employed for determining the content of paracetamol and tramadol. The linear model for determining content of paracetamol and tramadol were y = 0.9877x + 0.4663 (R^2=0.9959) and y = 0.9685x + 0.3401 (R^2=0.9875), respectively. The chemometrics model was applied in the content uniformity analysis of divided powder dosage form samples.

Keywords: chemometrics, compounding, paracetamol, tramadol, UV spectrophotometric

INTRODUCTION

Pharmacists as medical workers have a responsibility to ensure the safety and effectiveness of treatments for supporting the patient-oriented medication (1). Nowadays, pharmacists are facing a great challenge of drug compounding and dispensing to provide specific dose for each patient (2). Compounding can be described as several actions including drug preparing, mixing, assembling, packaging, and/or labelling for prescribed drugs in the practice of health professionals or other related fields such as research, teaching, or chemical analysis but not for commercial use (3).

Paracetamol and tramadol were pharmaceutical drugs which commonly prescribed in fixed-dose combination. Paracetamol works as a pain reliever for mild to moderate, while tramadol works as a pain reliever for moderate to severe pain (4,5). It has been reported that the combination of paracetamol and tramadol resulted in synergistic effect for relieving several inflammatory mechanisms (6). Other researches in the field of pharmacoeconomics proved that the combination of these two active pharmaceutical ingredients may improve the cost-effectiveness in patient medication (7,8).

In Indonesia, paracetamol and tramadol were commonly prescribed in the form of divided powder for reducing medication cost. One of the quality assessment method for compounded preparations was the determination of the content uniformity for each dose (9). In order to perform the content uniformity assessment, several analytical methods can be applied for analyzing content pharmaceuticals dosage form such as high-performance chromatography (10), thin-layer chromatography (11), and gas chromatography-mass spectrometry (12). In addition, chromatography can be applied to analyze not only pharmaceutical dosage form content but also food and natural product analysis (13,14). However, chromatographic techniques required high operational cost and time consuming for routine analysis in pharmacy or other public health services. Hence, it was important to develop a rapid, simple, effective, and low-cost analytical method to determine content both for paracetamol and tramadol in divided powder dosage form.

Chemometrics, the science of relating measurements resulted from a chemical system or process to the state of the system via employment of mathematical or statistical techniques, can be applied to solve this kind of problem (15). Spectroscopic techniques combined with multivariate calibration method namely partial least squares (PLS) was one of the chemometrics techniques which can be applied to determine the content of several compounds in a mixture matrices (16). Analysis of tablet containing paracetamol, propyphenazone, and caffeine was successfully performed using UV spectroscopic combined with PLS (17). Other research reported the analysis of paracetamol and tramadol in tablet dosage form using UV spectroscopic and multivariate calibration employed by commercial statistical software such as PLS Toolbox Solo, OriginPro, and Empower Enterprise (18). On the other hand, the R statistical software provided several statistical packages to perform chemometrics data analysis (19). R, open-source software with the GNU General Public License, offered a wide range of statistical tools by installing software packages related to each computational-statistics purpose (20). This study aimed to develop an alternative method to quantitatively analyze the content of paracetamol and tramadol in divided powder dosage form using UV spectroscopic coupled with multivariate calibration techniques. Two multivariate calibration techniques namely PLS and principal component regression (PCR) was performed and generated using the pls packages (21).

MATERIALS AND METHODS

Materials and Chemicals

The paracetamol and tramadol standard was obtained from PT. Dexa Medica, Indonesia. Analytical grade methanol was purchased from Merckmillipore and redistilled water was purchased from PT. Ikapharmindo Putramas. Samples of divided powder containing paracetamol and tramadol were formulated by the pharmacist in Yogyakarta, Indonesia.

Instrumentation and Software

A set of UV-Vis Spectrophotometer (Shimadzu, Japan) type UV 1800 equipped with quartz cuvette 1 cm (Hellma, USA), ultra-micro analytical balance RADWAG® series of UYA 2.3Y (max: 2.1 g, min 0.01 mg), and a set of Socorex® micropipettes was used in this study. The UV spectral data were exported to Excel (Microsoft) and converted into .csv formatted files for further data processing. Statistical analysis and multivariate calibrations were performed using R Studio software version 1.1.456 with pls packages. This software can be freely downloaded here https://rstudio.com/products/rstudio/download/.

Preparation of Standard Solutions

Accurate weight of 50.2 mg of paracetamol and 50.9 mg of tramadol were transferred into two separated 50 mL volumetric flasks for each compound followed by dilution with methanol into the volume. These solutions were labelled as paracetamol and tramadol stock solution.

Preparation of Calibration Set and Validation Set Solutions

Calibration set solution and validation set solution were prepared by mixing a synthetic combination of paracetamol and tramadol from the stock solutions to achieve 40 compositions of standards solution mixture as presented in Table 1. Each composition was scanned using UV spectrophotometer at the range of 230-400 nm. The absorbance for each composition was recorded with an interval of 2 nm. The obtained absorbance data for each wavelength were used for generating both calibration and validation models.

Preparation of Samples

Twenty single doses of divided powder dosage forms were weighted for each and homogenized accordingly. A 50 mg powder for every single dose was weighed, transferred into 50 mL volumetric flask, diluted with methanol into the volume, and filtered to remove drug excipients. A 500 μL filtrate was transferred into 10 mL volumetric flask followed by dilution into the volume.

Each sample was scanned using UV spectrophotometer at the range of 230-400 nm. The absorbance for each composition was recorded with an interval of 2 nm. The obtained absorbance data for each wavelength were used for determining the content of paracetamol and tramadol.

Spectroscopic Analysis and Multivariate Calibrations

The absorbance values of every single wavelength point achieved from the calibration and validation data sets were statistically analyzed using the R studio software. A statistical package of pls was employed to perform chemometrics data processing. This package has been downloaded and installed by using the function of install.packages("pls"). After successfully installed, this package was loaded by using the function of library(pls) before further statistical analysis. A couple of multivariate calibration models namely

PLS and PCR were generated for both paracetamol and tramadol. The built model multivariate calibration models were statistically evaluated by assessing several performances such as coefficient of determination (R^2), root mean square error of calibration (RMSEC), root mean square error of cross-validation (RMSECV), and root mean square error of prediction (RMSEP). Further, multivariate calibration model for each compound which resulted a value of R^2 near 1 and lower RMSEC, RMSECV, and RMSEP were chosen for content determination (22).

Content uniformity

The content uniformity of paracetamol and tramadol in the divided powder will be analyzed descriptively using the standard deviation (SD) values of each sample group with the following equation:

$$SD = \sqrt{\frac{\sum(x-\bar{x})^2}{N-1}}$$

The divided powder has good uniformity if all of the content of paracetamol and tramadol are included in the average susceptible plus minus standard deviation.

RESULTS AND DISCUSSION

Spectral Analysis

Spectroscopy analysis for paracetamol and tramadol was initially carried out by scanning UV spectra of paracetamol, tramadol, and synthetic mixture containing these two compounds. Figure 1 presented the UV spectra profile of paracetamol, tramadol, and mixed standards. Chemical structure for both paracetamol and tramadol was also depicted. The overlaid UV spectra showed that paracetamol and tramadol absorbed UV radiation and resulted in different spectra characteristics (23). However, it was difficult to analyze the binary mixture of different compounds with conventional spectroscopy method simultaneously (24). The employment of chemometrics techniques with pattern recognition algorithm was needed to overcome this problem (25).

Multivariate Calibration

Multivariate calibration models generated in this study were PCR and PLS. All the calibration models were built using R studio software (19) by exploiting one statistical package called pls (26). Both PCR and PLS were models for multivariate calibration, the difference between them was lied in the predictor. PCR works in reducing the number of predictor variables by using their first few latent variables chosen by cross-validation technique rather than the original variables, while PLS regression employs the linear combinations of the predictor variables rather than the original variables (22,27).

Table 2 presented the performance of PCR and PLS for predicting the content of paracetamol and tramadol in calibration set solutions. Parameters for evaluating the performance of calibration model was shown as the value of R^2, RMSEC, and RMSECV. Table 3 presented statistical parameters resulted from the validation data set evaluation. Model equations of PCR and PLS both for paracetamol and tramadol were shown along with the value of R2, RMSEP, and predicted residual error sum of squares (PRESS). In calibration data evaluation, PCR techniques for paracetamol and tramadol resulted in more satisfying data compared to PLS. On the other hand, in validation data evaluation the best results for paracetamol and tramadol were obtained from PLS techniques. With the many candidate models, it was not surprising if selecting the best model cannot be an easy task (28). In this study, the multivariate calibration resulted from PLS technique for paracetamol and tramadol was chosen since the values of several performances in calibration model were not significantly different and the consideration of validation models that generated to establish the agreement between the predictions and the observations for subsequent analysis (29).

PLS model for paracetamol and tramadol were built using eight principal components. Figure 2 depicted that the lowest RMSECV for these two compounds were obtained at eight components. This condition indicated that the cross-validation model has less error since the RMSECV refers to the uncertainty of cross-validation models (30). PLS calibration and validation model for determining the content of paracetamol and tramadol were presented in Figure 3. The linear model for determining content of paracetamol and tramadol were $y = 0.9877x + 0.4663$ ($R^2=0.9959$) and $y = 0.9685x + 0.3401$ ($R^2=0.9875$), respectively.

The observation of regression coefficient plots (Figure 4) provided useful information on important wavelengths which were considered in the quantitative determination of paracetamol and tramadol. It can be interpreted that several peaks and valleys at certain wavelengths significantly contributed in process of generating the multivariate calibration model (16). By this worthy approach, it can be rationally explained that the λmax of paracetamol (245 nm) and tramadol (272 nm) resulted in high peak profiles in regression coefficient plots.

Content Uniformity Analysis

Table 4 presented the content uniformity assessment of paracetamol and tramadol in 20 divided powders. The data shows that the content of both paracetamol and tramadol in divided powders have presented no uniformity statistically. The divided powders should have content uniformity since it was closely related to the dose of the drug taken by the patient each sac (31).

Several factors may affect the uniformity of the content of preparation, that are the lack of compounding skills, no indicators of homogeneity in the powder mixture, powder mixing techniques are not optimal, and the ability to divide the powder is not good. Some alternatives that can be done to improve the uniformity of the content are by adding an indicator of homogeneity to the mixture and weighing one by one the divided powder, especially if the drug has a narrow therapeutic index (32).

CONCLUSION

A UV spectrophotometric coupled with multivariate calibration method for simultaneous determination of paracetamol and tramadol in divided powder dosage form has been successfully conducted. PLS was chosen as multivariate calibration technique for determining both paracetamol and tramadol. It can be concluded that divided powder dosage forms compounded by the pharmacist in Yogyakarta, Indonesia do not have a good uniformity in content both paracetamol and tramadol.

This developed method was rapid, simple, low cost, and effective for routine analysis of divided powder dosage form. However, it is important to redevelop the multivariate calibration models for different formulation.

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TABLES AND FIGURES

Table 1: Calibration and validation data sets information for model selection and statistical analysis for determining content of paracetamol (PCT) and tramadol (TRM)

Items	Data sets	
	Calibration	Validation
Number of mixture standards	25	15
PCT Concentration (μg/mL)		
Mean	29.056	25.068
Range	11.5 - 55.22	11.04 - 55.22
TRM Concentration (μg/mL)		
Mean	10.608	9.977
Range	2.55 - 29.52	2.04 - 25.45
Multivariate calibration models	PCR PLS	PCR PLS
Evaluated parameters for model selection	R^2 RMSEC RMSECV*	R^2 RMSEP

Note: PCT: paracetamol; TRM: tramadol; * cross validation was performed using leave one out technique

Table 2: The performance of principle component regression (PCR) and partial least squares (PLS) for predicting the content of paracetamol and tramadol in calibration set solutions

Compounds	Multivariate calibrations	Calibration			
		Number of components	R^2	RMSEC	RMSECV
Paracetamol	PCR	10	0.9922	1.2192	1.195
	PLS	8	0.9908	1.3221	1.295
Tramadol	PCR	11	0.9843	0.8944	0.8763
	PLS	8	0.9843	0.8947	0.8766

Table 3: Statistical parameters resulted from validation data set evaluation

Parameters	Paracetamol		Tramadol	
	PCR	PLS*	PCR	PLS*
Equation	y = 0.9853x + 0.5588	y = 0.9877x + 0.4663	y = 0.9665x + 0.3614	y = 0.9685x + 0.3401
R^2	0.9958	0.9959	0.9869	0.9875
PRESS	14.1865	13.7036	8.9418	8.5155
RMSEP	0.9725	0.9558	0.7721	0.7535

Note: * indicated the chosen model of calibration for each compound

Table 4: The Content Uniformity Assessment of Paracetamol and Tramadol.

Parameter	Paracetamol	Tramadol
Lowest value	33.783 mg	12.180 mg
Highest value	57.130	24.470 mg
Mean	45.070 mg	15.291 mg
Standard deviation	4.684 mg	2.383 mg
Range	40.387 – 49.754 mg	12.908 – 17.674 mg
Data in range	16 samples	18 samples
Data out of range	4 samples	2 samples

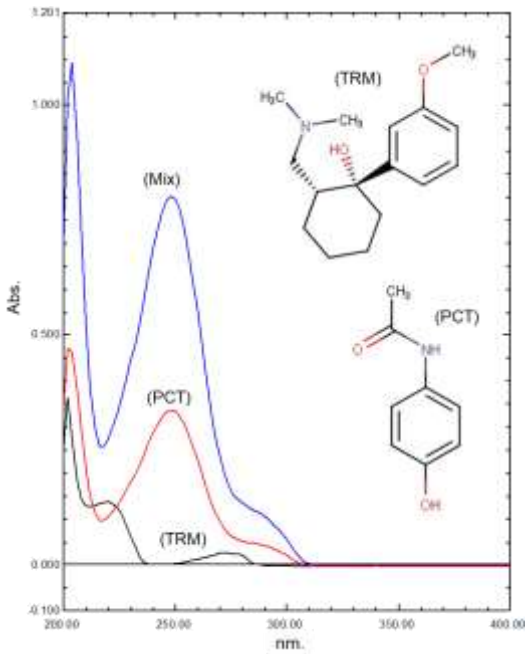

Fig.1: The chemical structure and UV spectra profile of paracetamol (PCT), tramadol (TRM), and mixed standards (Mix)

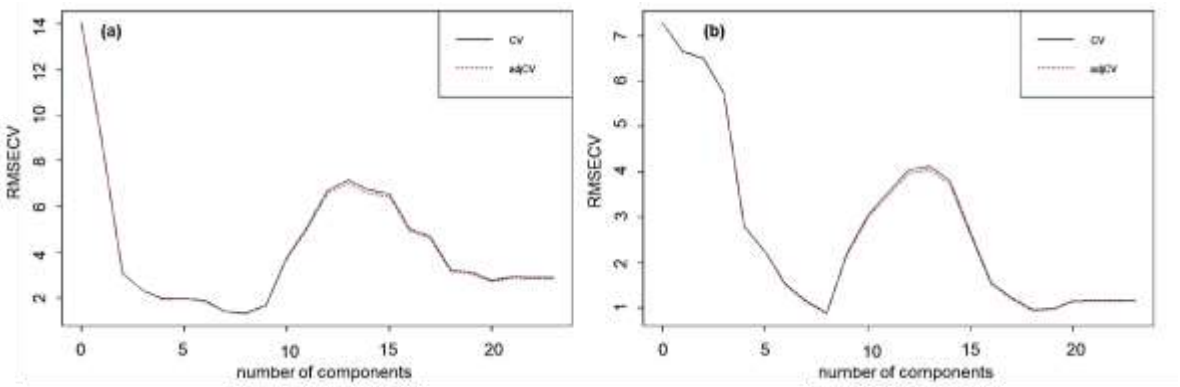

Fig.2: Number of components versus RMSECV for PLS calibration model for determination of paracetamol (a) and tramadol (b)

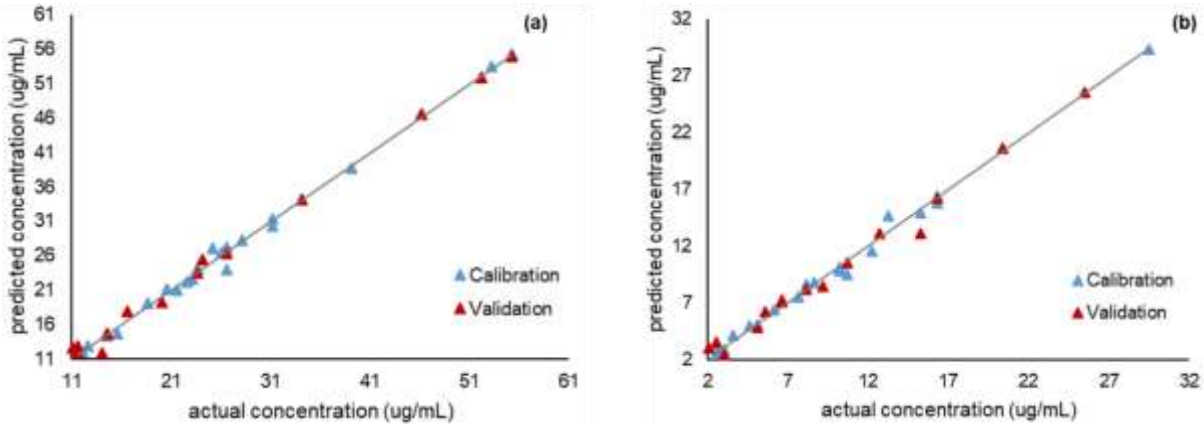

Fig.3: PLS calibration and validation model for determining content of paracetamol (a) and tramadol (b)

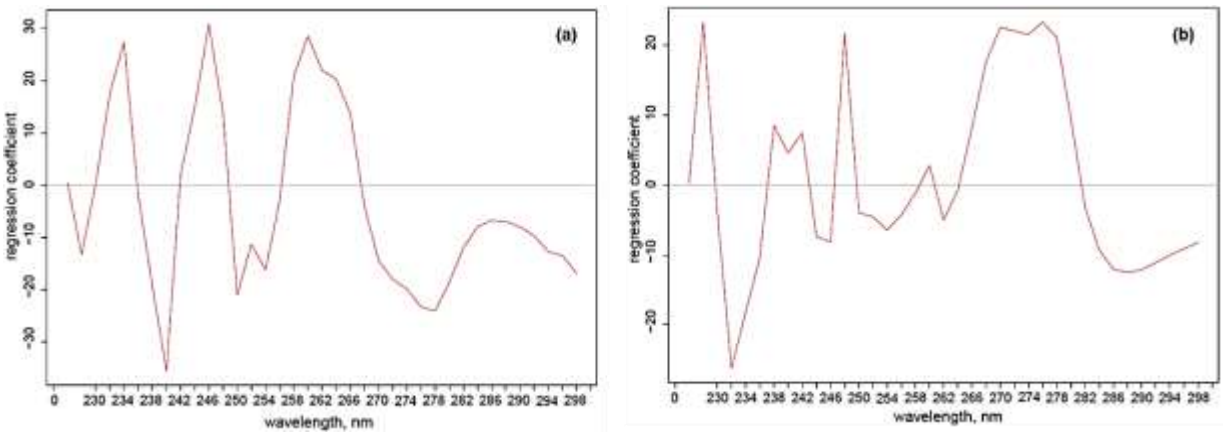

Fig.4: Regression coefficients versus wavelength of paracetamol (a) and tramadol (b)