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Short Communication

Simultaneous Determination of Metamizole, Thiamin and Pyridoxin Using UV-Spectroscopy in Combination with Multivariate Calibration

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Abstract
Purpose: Analysis of drugs in multicomponent system officially is carried out using chromatographic technique, however, this technique is too laborious and involving sophisticated instrument. Therefore, UV-VIS spectrophotometry coupled with multivariate calibration of partial least square (PLS) for quantitative analysis of metamizole, thiamin and pyridoxin is developed in the presence of cyanocobalamine without any separation step.
Methods: The calibration and validation samples are prepared. The calibration model is prepared by developing a series of sample mixture consisting these drugs in certain proportion. Cross validation of calibration sample using leave one out technique is used to identify the smaller set of components that provide the greatest predictive ability. The evaluation of calibration model was based on the coefficient of determination (R^2) and root mean square error of calibration (RMSEC).
Results: The results showed that the coefficient of determination (R^2) for the relationship between actual values and predicted values for all studied drugs was higher than 0.99 indicating good accuracy. The RMSEC values obtained were relatively low, indicating good precision. The accuracy and presision results of developed method showed no significant difference compared to those obtained by official method of HPLC.
Conclusion: The developed method (UV-VIS spectrophotometry in combination with PLS) was succesfully used for analysis of metamizole, thiamin and pyridoxin in tablet dosage form.

Introduction

Metamizole (MET), Thiamine (B_1) and Pyridoxin (B_6) are active pharmaceutical ingredients frequently combined and widely used to relieve pain complaints caused by neuritis and neuralgia, especially on severe pain.[1] Metamizole is pirazolon derivative having analgesic and antipiretic effects. It is commonly used to relieve acute pain.[2] Thiamine and Pyridoxin are neurotropic vitamins which play an important role in formation of energy metabolism needed by brain cells. The combination of MET with vitamin B complex (i.e. thiamine and pyridoxin) will increase the potential synergistic effect of analgesic-antipyretic.[3,4] The chemical structures of the studied drugs are shown in Figure 1.

Some analytical methods have been reported for the determination of MET, B_1 and B_6, either alone or in combination with other medicines in pharmaceutical products. Several analytical methods for determination MET such as electrochemical and electrophoretic,[5] reflectometric,[6] spectrophotometry,[7] HPLC[8] and liquid chromatography-mass spectrometry (LC/MS) for bioequivalence study.[9] Furthermore, several analytical methods for quantification B_1 and B_6 such as densitometry and spectrofotometry in combination with multivariate calibration,[10] capilary zone electrophoresis,[11] HPLC,[12,13] and LC/MS[14,15] have beed used. The combination of MET, B_1 and B_6 is comercially available in tablet dosage form, and the widely used analytical method is HPLC.[16] However, some of these methods are time consuming, involving some reagents, and requiring sophisticated instruments, therefore, UV-VIS spectrophotometry is continuously developed to overcome these difficulties.

Although low selectivity and sensitivity, the spectrophotometric techniques is the most interesting approach to be applied in pharmaceutical analysis owing to its simplicity. However, in many cases of the multicomponents assay in pharmaceutical preparation, UV-VIS spectrophotometric techniques showed useless results because of a high number of components and the extensive spectral overlapping,[17] therefore, the chemometrics technique is applied to resolve this problem. [18]

Currently, the application of chemometric techniques, especially multivariate calibrations are playing a very important role in the multicomponent analysis of pharmaceutical mixtures.[19] Some multivariate calibrations such as principal component regression (PCR), stepwise

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multiple linear regression (SMLR), and partial least-squares (PLS) are the most adopted multivariate methods in pharmaceutical analysis and are frequently used for instrumental methods without separation techniques like ultraviolet and infrared spectroscopies.[20,21] UV-VIS Spectrophotometry in combination with multivariate calibration of partial least square has been used for analysis of paracetamol, phenylephrine and chlorpheniramin in tablet dosage form[22] and simultaneous analysis of riboflavin, thiamine, nicotinamide and pyridoxine in pharmaceutical formulations.[23] However, there is no reports regarding analysis of metamizole (MET), thiamine (B_1) and pyridoxin (B_6) simultaneously using UV-VIS spectrophotometry and multivariate calibration. Therefore, the objective of this study was to determine MET, B_1 and B_6 in synthetic mixture and in tablet formulation using UV-VIS spectrophotometry in combination wiith multivariate calibration.

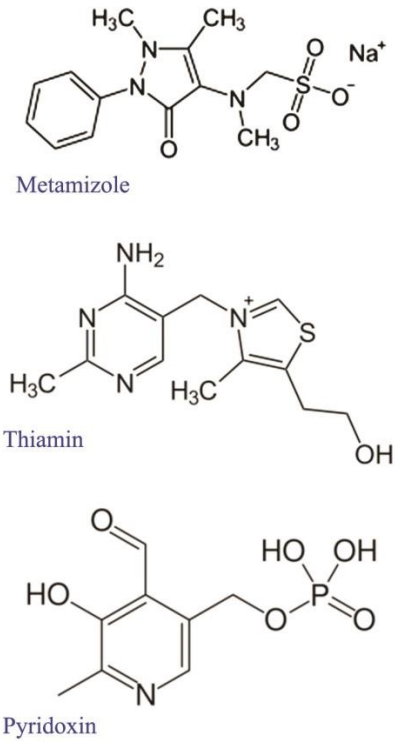

Figure 1. The chemical structure of Metamizole, Vitamin B_1 and Vitamin B_6.

Materials and Methods

The standards of metamizole (MET), Thiamin (B_1) dan Pyridoxin (B_6) 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 (Dololicobion, Berlico Mulia Farma Yogyakarta, Indonesia)was obtained from pharmacy in Yogyakarta, labelled to contain 500 mg, 50 mg, 100 mg and 100 µg of Metamizole, vitamin B_1, vitamin B_6 and vitamin B_{12} respectively, in each tablet.

Instrumentation and Software

Absorption measurements were done using Shimadzu UV Spectrofotometer UV-1800, and spectral data were exported to Excel and manipulatd using Minitab software version 16 (Minitab Corp., USA). All measurements were carried out using 1 cm quartz cells over the wavelength range of 200 – 400 nm with 2 nm interval. Shimadzu LC 20 AD was used for high performance liquid chromatographic measurement.

Chromatographic condition

Stationary phase, a C18 column (150 × 4.6 mm, 5 µm) with a mobile phase consisting of a solution of PIC: methanol: glacial acetic acid in the ratio of 700: 300: 4 (v/v/v) was used during HPLC analysis. PIC solution composed of 0.522 g of sodium pentanesulfonate and 0.404 g of sodium *n*-heptanesulfonate, dissolved in 700 mL of water in a 1000 mL volumetric flask. The flow rate of mobile phase was set at 1.0 mL/min and UV detector was set at 275 nm. The analytical method above was adopted from the National of Drug and Food testing Center of Republic Indonesia code 28/OB/01 for simultaneous analysis of metamizole, vitamin B_1 and vitamin B_6 in tablet dosage forms.

Preparation of standard solution

The standard solutions were prepared freshly in hidrochloric acid 0.1 N and used for preparing calibration samples (20 samples) and validation samples (10 samples). The composition of calibration and validation samples are shown in Table 1 and 2, respectively. Each solution mixture was scanned using UV Spectrophotometer at 200 – 400 nm. Each 2 nm, their absorbance were recorded and used for the optimization of calibration models.

Analysis of metamizole, vitamin B1 and vitamin B6 in tablet dosage forms using UV spectrophotometry

Twenty tablets were taken and subjected to weight homogenity test. The tablets are chrushed until homogenous and an amount of powder equivalent to one tablet is taken and dissolved with 0.1 N hydrochloric acid until 100 mL. The solution is shaken vigorously for 30 minutes, and subsequently diluted to obtain concentration of 40 µg/mL MET, 4 µg/mL Vitamin B_1, and 8 µg/mL vitamin B_6. The solution is filtered using Whatman paper, and the supernatant is taken and subjected to spectrophotometric measurement as described above. The concentration of metamizole, vitamin B_1 and vitamin B_6 in tablet dosage forms is calculated based on the optimized calibration model. All determinations were performed in six times.

Analysis of metamizole, vitamin B1 and vitamin B6 in tablet dosage forms using high performance liquid chromatography

Standard and sampel solutions were made by weighing an equivalent of 10 mg MET in 50 mL volumetric flask and diluting it with acetic acid 0.5%. Then, the solution

was shaken vigorously for 30 minutes. The solution is filtered using Whatman microfilter Ø 0.45 µm, and the supernatant is taken and subjected to HPLC measurement as described above. The concentration of MET, vitamin B_1 and vitamin B_6 in tablet dosage forms is calculated based on single point calibration. All determinations were performed in six times.

Table 1. The composition of synthetic mixture consisting of Metamizole (MET), Thiamin (B_1), Pyridoxin (B_6), and Cyanocobalamin (B_{12}) used in calibration samples.

No of samples	Metamizole (µg/mL)	B_1 (µg/mL)	B_6 (µg/mL)	B_{12} (µg/mL)
1	37	5	4	0,19
2	9	8	2	0,10
3	19	14	3	0,04
4	14	19	8	0,03
5	12	12	9	0,14
6	18	5	15	0,05
7	16	9	18	0,15
8	22	10	11	0,07
9	48	4	5	0,19
10	10	10	17	0,16
11	28	2	10	0,11
12	15	13	12	0,16
13	29	6	14	0,02
14	45	6	7	0,11
15	41	8	19	0,07
16	24	2	10	0,09
17	31	7	12	0,03
18	35	16	19	0,17
19	27	3	16	0,07
20	43	6	9	0,01

Table 2. The composition of synthetic mixture consisting of Metamizole (MET), Thiamin (B_1), Pyridoxin (B_6), and Cyanocobalamin (B_{12}) used in validation samples.

No of samples	Metamizole (µg/mL)	B_1 (µg/mL)	B_6 (µg/mL)	B_{12} (µg/mL)
1	23	1	10	0,02
2	44	3	7	0,05
3	8	9	8	0,09
4	25	8	11	0,17
5	21	18	12	0,04
6	13	6	6	0,09
7	35	8	7	0,07
8	9	10	12	0,15
9	25	15	15	0,17
10	22	9	20	0,04

Results and Discussion

Figure 2 showed the overlay of absorption spectra of metamizole (MET), thiamin (B_1), pyridoxin (B_6) dan cyanocobalamin (B_{12}) in 0.1 N hidrochloric acid at wavelength of 200-400 nm. A clear extensive overlapping among the spectra curve was observed which prevented to determine MET, B_1 and B_6 simultaneously using ordinary UV-VIS spectrophotometry. In addition, vitamin B_{12} is usually present in the concentration level of 500 – 1000 times lower than the other components, and at this ratio, vitamin B_{12} does not have a considerable absorbance at the same wavelenght range. To overcome the overlapping spectra among MET, B_1 and B_6, the quantitative analysis was performed with the aid of partial least square (PLS) calibration. This techniques is done in three step, namely calibration, validation and prediction of unknown samples.[24]

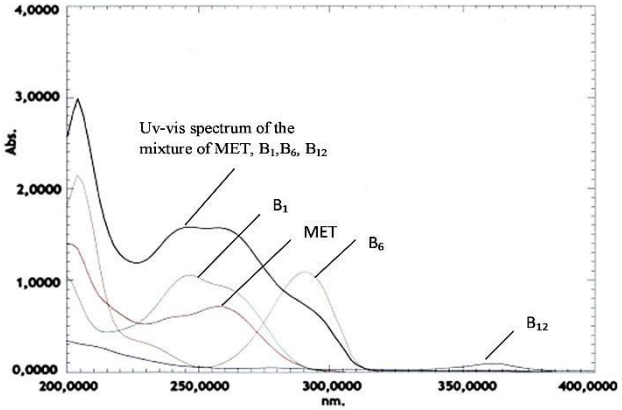

Figure 2. The overlay of UV spectra of Metamizole, Vitamin B_1 and Vitamin B_6.

The multivariate calibration of PLS modelling for quantitative analysis of MET, B_1 and B_6 starts with the evaluating UV spectra between the mixtures of studied drugs and those of dosage form at the same concentration. In order to be succesfull during PLS modelling, both spectra were recommended to be similar as shown in Figure 3. Then some wavelengths were optimized during PLS modelling to obtain the wavelengths which able to provide the best correlation between actual value of MET, B_1 and B_6 and its predicted value. Finally, the wavelength of 230 – 320 nm was prefered for quantification of MET, B_1 and B_6 simultaneously because of its ability to provide the highest values of coeficients of determination (R^2) and the lowest values of error expressed as root mean square error of calibration (RMSEC). The R^2 values obtained for the correlation between actual value and predicted value of MET, B_1 and B_6 as determined using UV spectrofotometry at 230 – 320 nm are high, namely 0.9999, 0.9998, and 0.9999 respectively, for MET, B_1 and B_6. Meanwhile, the RMSEC values obtained are relatively low, i.e. 0.1035 % (MET), 0.0540 % (B_1) and 0.0428 % (B_6).

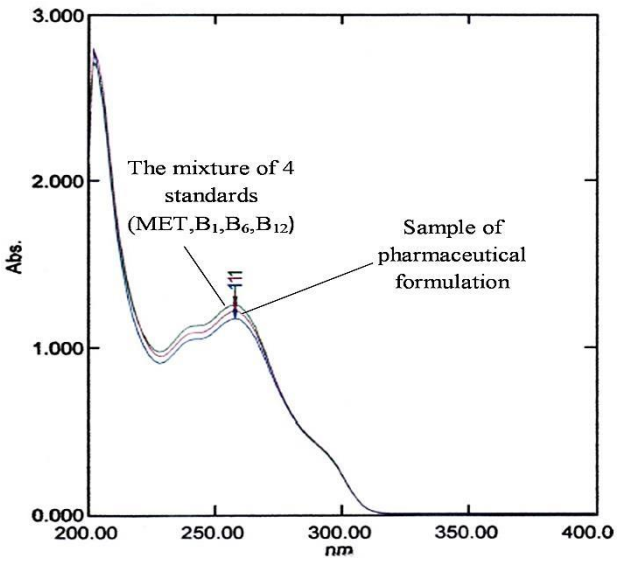

Figure 3. The UV spectra overlay of the drug mixture (Metamizole, Vitamin B1 and Vitamin B6) and formulation containing these drugs.

One of the potential disadvantages when analyst use multivariate calibrations is over-fitting of the regression model, which 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 data sets with similar material.[25] To overcome this problem, we used cross-validation of calibration samples using "leave-one-out" technique. The first sample from a set of calibration samples are omitted from PLS model and the remaining samples are used to make new developed PLS model. Then the removed sample is calculated using the new developed PLS model. This prosedure is repeated, leaving each calibration sample out in turn. The sum of the squares of these differences is called predicted residual error sum of square (PRESS). The smaller PRESS value means the better predictive power of model.[26] Moreover, cross-validation is used to identify the smaller set of components that provide the greatest predictive ability, and Minitab software selects the model with the number of components that produces the highest predicted R^2 and the lowest PRESS. The PRESS value obtained are 5.22 (MET), 1.262 (B_1) and 0.6189 (B_6). Meanwhile the predicted R^2 values are 0.9981, 0.9968, and 0.9989 respectively for MET, B_1 and B_6. The UV spectra in combination with PLS model was subsequently used to predict the level of validation samples. The prediction performance was assessed by R^2 and root mean square error of prediction (RMSEP) values; the closer R^2 to one and the lower RMSEP indicate the better prediction model of new samples. Besides the high value of R^2 and low value of RMSEP indicated good recovery and less error. The R^2 and RMSEP values obtained are 0.999 (0.3993 %); 0.999 (0.1926 %); 0.999 (0.1434 %) respectively, for MET, B_1 and B_6.

Furthermore, the developed method was used for determination of MET, B_1 and B_6 in tablet dosage form. The results obtained by UV spectrophotometry are compared with those using official methods (HPLC). The level of MET, B_1 and B_6 obtained by UV spectrophotometry in combination with PLS and by HPLC are shown in Table 3. After t test and F test, it is known that results obtained by UV spectrophotometry in combination with PLS and HPLC showed no significant difference ($P > 0.05$). It can be concluded that UV spectrophotometry can be an alternative technique for determination of MET, B_1 and B_6 in tablet dosage form simulteneously without separation ttechnique.

Table 3. The level of Metamizole, B_1 and B_6 of tablet dosage form obtained by UV spectrophotometry in combination with PLS and by HPLC

No sample	Metamizole (%)		B1 (%)		B6 (%)	
	UV	HPLC	UV	HPLC	UV	HPLC
1	96.95	94.87	102.24	99.00	101.16	99.27
2	97.03	95.73	101.42	101.16	98.07	98.34
3	96.47	96.61	97.47	100.02	97.58	98.65
4	96.32	95.95	99.30	100.69	96.43	99.56
5	95.55	96.30	100.44	97.18	99.25	98.17
6	96.27	96.93	101.93	98.05	98.17	99.77
Average	96.43	96.06	100.47	99.35	98.44	98.96
SD	0.54	0.73	1.82	1.55	1.61	0.66
RSD	0.56	0.76	1.81	1.56	1.64	0.67
$T_{calculated}$ (t_{table})	0.331 (1.812)		0.381 (1.812)		0.242 (1.812)	
$F_{calculated}$ (F_{table})	1.828 (9.364)		1.374 (9.364)		5.891 (9.364)	

Conclusion

The use of UV spectrophotometry and PLS calibration model for the simultaneous analysis of MET, B_1 and B_6 can be an alternative technique for quality control of the pharmaceutical formulations without separation step. The results obtained with UV spectrophotometry are comparable with those obtained using HPLC. The developed method is simple and is not time consuming.

However, if the composition of matrix is different, the new PLS calibration model should be developed.

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Ethical Issues
Not applicable.

Conflict of Interest
The authors declare that they have no conflict of interest.

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