Original Article

Mahidol University Journal of Pharmaceutical Science 2011; 38 (3-4), 23-33

Chemometrics-Assisted UV Spectrophotometric Method for Determination of Acetaminophen and Chlorzoxazone in Tablets

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Abstract

Chemometrics-assisted spectrophotometric approaches, principle component regression (PCR) and partial least square regression (PLS-1), were employed for determination of acetaminophen and chlorzoxazone in tablets. Two sets of standards mixtures, calibration set and test set, were prepared. The set of calibration mixtures were created by using a central composite design (CCD). The UV absorbance spectra of the resulted samples were subjected to partial least square regression (PLS-1) and principal component regression (PCR). The optimum regression models were used to determine the test set solutions for validation the models. An HPLC method was developed and also employed for comparison. The developed HPLC method, PLS-1 and PCR models were used to determine acetaminophen and chlorzoxazone in tablets. The determination results showed that the data obtained from PLS-1 and PCR models were not significantly different with those obtained from HPLC method at 95% confidence limit.

Key words: Chemometrics, PCR, PLS-1, Acetaminophen, Chlorzoxazone

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INTRODUCTION

Acetaminophen (paracetamol, ACP) is widely used in normal and deep pain and chlorzoxazone (5-chloro-2-hydroxybenzoxazole, CZX) is a muscle relaxant which interacts to central nervous system. Combination of ACP and CZX has been commercial available in tablets dosage form. Determination of these substances in tablet, according to the United States Pharmacopoeia (USP) 32[1] and other studies[2-3], were performed by HPLC method. Analysis of ACP and CZX in combinations could not be performed by direct UV spectrophotometer without chromatographic separation since the overlapping of their UV spectra. Application of chemometrics approach with spectrophotometric data may overcome this limitation.

Multivariate calibration is a chemometrics method which has been employed for determination of drugs in combination dosage forms including tablets[4-21]. This study aims to introduce alternative analytical procedure based on chemometrics-assisted spectrophotometric method for analysis of ACP and CZX in tablet. An HPLC method was also developed and validated for simultaneous determination of intend compounds. The tablet samples were assayed with the optimum chemometrics-assisted spectrophotometric method and developed HPLC method for comparison. In addition, this work is the first application of multivariate calibration methods, principle component regression (PCR) and partial least square regression (PLS-1), for determination of ACP and CZX combination in tablets.

MATERIALS AND METHODS

Materials

Standard acetaminophen was purchased from Anqiu Luan Phamaceutical Co.Ltd., China and chlorzoxazone was obtained from Sigma, Germany. Acetonitrile and methanol (HPLC grade) were purchased from Lab-Scan, Thailand. Potassium dihydrogen phosphate (Analytical grade) was obtained from Fluka, Switzerland.

Apparatus and software

The absorbance spectra were recorded by a Shimadzu (UV-160A) UV-Vis spectrophotometer combined with a 1 cm quartz cell. Chromatography was performed on a high-performance liquid chromatography system (Shimadzu corporation, Kyoto, Japan) consisting degasser DGU-12A, liquid chromatograph LC-10 AD, communications bus module CBM-10A, UV-Visible detector SPD-10A and data processing (class LC-10). The analytical column was a Gemini-NX C18, 250 × 4.6 mm i.d., 5µm (Phenomenex, USA). Manual injection was made by using a Rheodyne model 7725 injector with a 20-µL loop. Unscrambler® program was purchased from Charpa Techcenter Co., Ltd. (Bangkok, Thailand). Data analysis, PCR and PLS-1 calibrations were performed by Unscrambler® program.

Chromatographic system

The mixture of 25 mM phosphate buffer, pH 4.5 and methanol (45:55, v/v) was utilized for the elution of ACP and CZX. The occurrence of two compounds was detected by UV detector at 281 nm. The flow rate of mobile phase was 1.0 mL/min.

Standard preparations

Stock standard solutions of ACP and CZX at the concentration of 1 mg/mL were separately prepared by dissolving accurately weighed amount of the drugs in methanol. The working standard solutions of two drugs were prepared by dilution of their stock solutions with 0.1N HCl until the final concentration of 0.1 mg/mL for each drug was obtained.

Preparation of calibration curves for HPLC method

The standard mixtures of ACP (2.4–24.0 µg/mL) and CZX (2.0-20.0 µg/mL) were prepared in 10 mL volumetric flasks by using their working standard solutions. All standard mixtures were adjusted to volume 0.1N HCl. Each standard mixture solution was injected into chromatographic system described above. The calibration curve of each drug was separately plotted

between concentrations (x-axis) versus corresponding peak areas (y-axis).

Sample preparation

Twenty tablets were weighed and finely powdered. A portion of the powder equivalent to about 300 mg of ACP and 250 mg of CZX was accurately weighed and transferred to a 50-mL volumetric flask. Methanol was added to dissolve and adjust to volume. The solution was sonicated and then filtered through 0.45 μm nylon membrane. The filtrate was diluted with 0.1N HCl until the final concentrations of 9.6 μg/mL and 8.0 μg/mL were obtained for ACP and CZX, respectively. This solution was filtered by using 13 mm, 0.45 μm nylon syringe filter before injecting into the HPLC column.

Development and validation of HPLC method

Standard mixture solution containing 9.6 μg/ml of ACP and 8.0 μg/ml of CZX was employed for HPLC method development. Mobile phase conditions such as type and concentration of organic solvents, mobile phase pH, were studied to obtain a suitable separation condition. Performance characteristics selected for method validation were linearity, accuracy and precision.

Linearity

Linearity was evaluated in the concentration range of 2.4-24.0 μg/mL for ACP and 2.0-20.0 μg/mL for CZX. The data were analyzed by least-squares linear regression method.

Accuracy

Accuracy of the developed method was studied by standard addition. The sample containing 4.8 μg/mL of ACP and 4.0 μg/mL of CZX was added to standard mixtures of ACP and CZX. Three concentrations of drugs were employed in this study, 4.8, 9.6 and 14.4 μg/mL for ACP and 4.0, 8.0 and 12.0 μg/mL for CZX. Three replicates were performed for each concentration level. The accuracy of the method was expressed in term of recovery percent between amount of standard added and amount of standard found.

Precision

Precision was investigated for both intra-day and inter-day precision. For intra-day precision, three concentration levels of standard mixtures (4.8, 9.6 and 14.4 μg/mL for ACP and 4.0, 8.0 and 12.0 μg/mL for CZX) were analyzed. Six determinations were done for each concentration on the same day. All solutions were injected in three replicates. For inter-day precision, the same three different concentrations as for intra-day were studied on three different days and each concentration was triplicately injected. The precision of the method was expressed as the percentage of relative standard deviation (%RSD).

Chemometrics experiment

One component calibration

To find the linear dynamic concentration range of each drug, one component calibration was performed. Linear dynamic ranges were studied in the concentration range of 2.4-19.2 μg/mL for ACP and 2.0-16.0 μg/mL for CZX. Absorbance values were recorded at λ_{max} of each drug (243 nm for acetaminophen and 281 nm for chlorzoxazone) in 1-cm quartz cell and used 0.1N HCl as blank. Linear dynamic range for each compound was determined by least-square linear regression of concentration and the corresponding absorbance.

Binary standards solutions

Two sets of standard solutions, calibration set and test set were prepared. According to Table 1, 12 mixtures solutions and 9 mixtures solutions were used in calibration set and test set, respectively. The concentrations of calibration set were selected by mean of central composite design (CCD)[21] and those of test set were randomly selected.

Table 1. Composition of calibration set and test set

Mixture	Calibration set		Test set	
	ACP (μg/mL)	CZX (μg/mL)	ACP (μg/mL)	CZX (μg/mL)
1	0.0	8.0	3.0	2.5
2	19.2	8.0	12.0	10.0
3	9.6	0.0	9.6	8.0
4	9.6	16.0	6.0	5.0
5	2.8	2.3	14.0	7.0
6	2.8	13.6	19.2	2.0
7	16.4	13.6	3.0	16.0
8	16.4	2.3	5.0	14.0
9	9.6	8.0	8.0	12.0
10	9.6	8.0		
11	9.6	8.0		
12	9.6	8.0		

PCR and PLS-1 models building

The solution in calibration set and test set were measured the absorbance data in the wavelength interval of 200-400 nm. The absorbance data of calibration set were then subjected to the Unscrambler® program for PCR and PLS-1 models building. For validation the PCR and PLS-1 models, the resulting models were used to assay the concentrations in test set which were not contributed in models building.

RESULTS AND DISCUSSION

HPLC Method development

Methanol and acetonitrile were employed for HPLC method development. Concentrations of organic solvents were also studied. The suitable organic solvent and concentration was methanol 55 % by volume. The buffer, 25 mM potassium phosphate, had an advantage over water in this study. The pH of 25 mM phosphate buffer was investigated. The result showed that 25 mM phosphate buffer, pH 4.5 was the optimum pH for separation of ACP and CZX in this study. The retention times obtained from the optimum chromatographic condition were about 3.0 min for ACP and 7.8 min for CZX. The chromatogram of standard mixture was illustrated in Figure 1.

Validation of the method

Linearity

The linear dependence of the peak area and concentration of ACP was evaluated in the concentration range of 2.4-24.0 μg/mL. Excellent linearity was obtained over the entire concentration range and correlation coefficient (R^2) was greater than 0.999. The relationship between concentration of ACP (x-axis) and peak area (y-axis) was y = 12,231x – 6,445. For CZX, the calibration curve was linear over the concentration range of 2.0-20.0 μg/mL with the correlation coefficient (R^2) greater than 0.999. The relationship between concentration of CZX (x-axis) and peak area (y-axis) was y = 28,632x -2,089. Linearity graphs were shown in Figure 2.

Accuracy

Accuracy was represented by the recovery percent of standard found and standard added to the sample. The mean value at each concentration level is shown in Table 2. The average recovery percentages of ACP over the concentration range of 4.8-14.4 μg/mL was 99.9 to 100.5 % and the average recovery percentages of CZX over the concentration range of 4.0-12.0 μg/mL was 98.9 to 99.7 %. Good recoveries of both drugs implied that the developed HPLC method was suitable for separation of desired drugs in tablets.

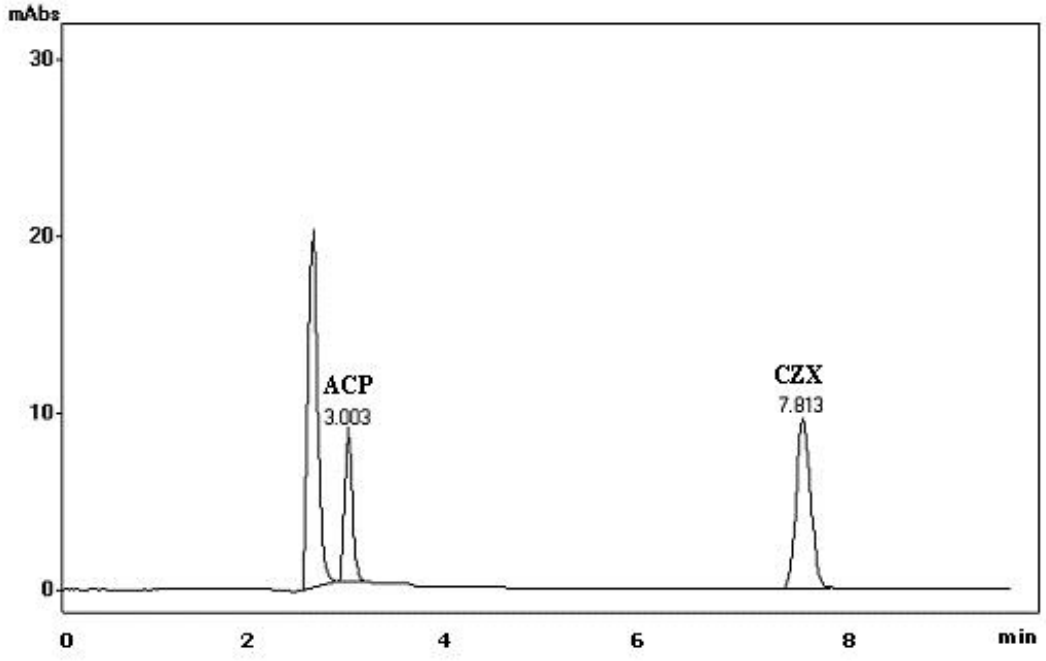


Figure 1. Chromatogram of ACP and CZX obtained from the optimum chromatographic Condition

Precision

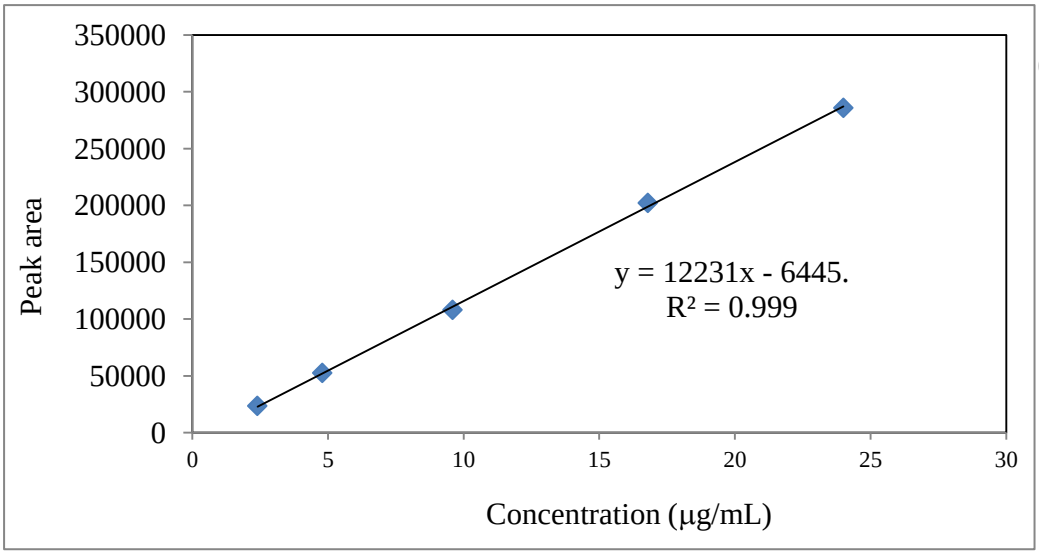
As summarized in Table 2, the intra-day precision of determination, as indicated by the relative standard deviation (RSD) value, for ACP ranged from 0.5 to 1.1% while the intra-day precision of CZX ranged from 0.4 to 1.2 %. The inter-day precision for both drugs were less than 1.5% over the entire concentration range 4.8-14.4 μg/mL for ACP and 4.0-12.0 μg/mL for CZX. These results showed that the proposed HPLC method yielded a satisfactory precision for analysis of ACP and CZX.

Chemometrics results

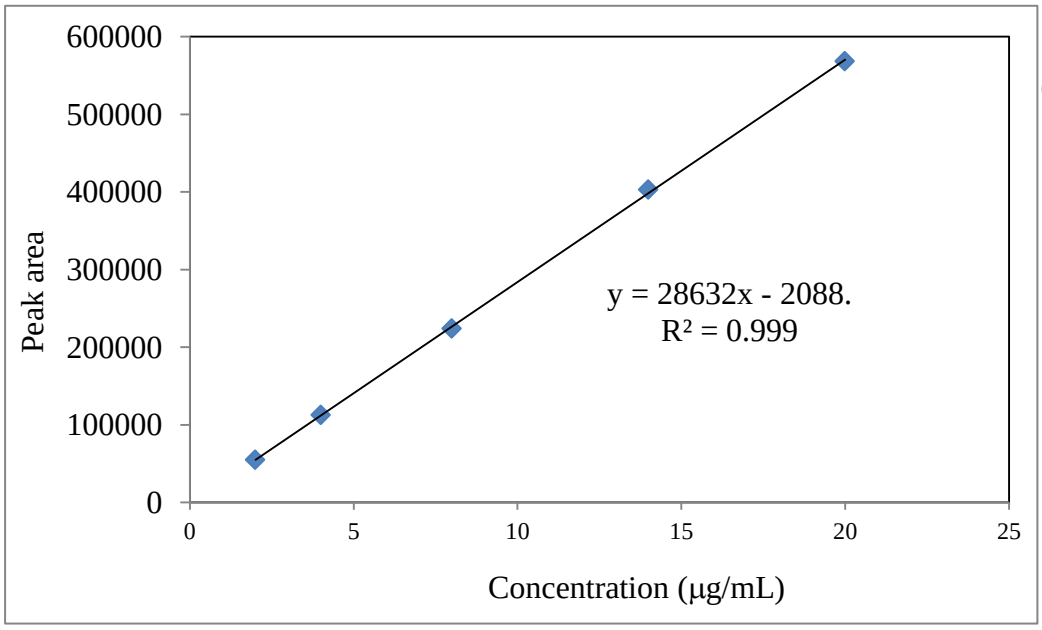
The UV spectra of ACP and CZX partially overlap in the UV region (Figure 3), this was not allowed for simultaneous determination of these compounds by conventional univariate calibration methods. Therefore, multivariate calibration methods such as PCR and PLS-1 were employed for their analysis.

The standard solutions used in the multivariate calibration methods are mixtures of analytes. There are some cautions should be considered in preparing of these standard solutions[22]. The first one is that the concentration of each analyte must be in its linear dynamic range. The concentration of the analytes in the calibration samples (Table 1) must be orthogonal. The absorbance of any mixture should not exceed the maximum absorbance reading of the instrument, and the concentration of the test set should be the same range as that of the calibration mixtures.

The resulted univariate calibration equations for the analytes at λ_{max} (243 and 281 nm for ACP and CZX, respectively) were linear in the ranges of 2.4-19.2 μg/mL for ACP and 2.0-16.0 μg/mL for CZX (Figure 4). To prevent obtained solutions with overload absorbencies, the concentrations of ACP and CZX in the mixtures were taken as 0-19.2 and 0-16.0 μg/mL, respectively. The composition of the test samples (Table 1) was selected randomly according to the linear dynamic ranges.



(2a)



(2b)

Figure 2. Linear response plots of ACP (2a) and CZX (2b)

Table 2. Accuracy and precision results of the developed HPLC method

Concentration (µg/mL)	% Recovery (Average, n = 3)	Intra-day precision (% RSD, n = 3)	Inter-day precision (%RSD, n = 3)
ACP			
4.8	100.1	1.1	1.2
9.6	99.9	0.6	0.5
14.4	100.5	0.5	0.4
CZX			
4.0	99.7	1.5	0.6
8.0	98.9	0.4	0.4
12.0	99.4	0.5	0.6

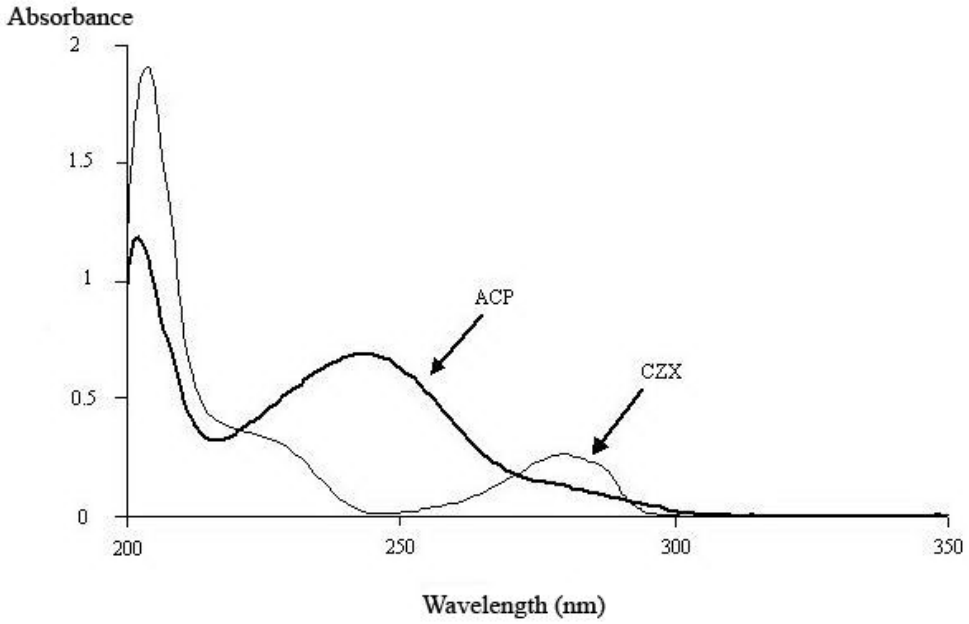


Figure 3. UV spectra of ACP and CZX

Results of PCR and PLS-1 analysis

The PCR and PLS-1 models were developed by Unscrambler® program. Model development was performed by using calibration standards. Leave-one-out cross-validation (LOO-CV) was used to validate PCR and PLS-1 models in model development and obtaining optimum latent variables (number of factors) of model. The parameters of optimum models were illustrated in Table 3. The resulted models were also validated by prediction the concentrations of analytes in a separate test set which was not used in the model development. The results of prediction and the percentage of recoveries are represented in Table 4. As observed, there was good agreement between the predicted (calculated) and actual concentrations of drugs. The mean recoveries for ACP and CZX were 101.1 % and 100.5 % for PCR models and 100.9 % and 100.4 % for PLS-1 models, confirming the high prediction power of the resulted models. The results obtained from PCR and PLS-1 models were not significantly different at 95% confidence limit.

Comparison of the PCR and PLS-1 models with HPLC method

In order to compare the results of the proposed PCR and PLS-1 models for determination of ACP and CZX in tablets, the HPLC method was also employed. The same sample solutions used for PCR and PLS-1 models were applied by HPLC method. The determination results of PCR, PLS-1 and HPLC methods are presented in Table 5. The determination data were expressed in term of percent labeled amount. The results showed that the average percent labeled amount obtained from PCR and PLS-1 models were not significant different from those obtained from HPLC method with the confidence limit of 95%.

CONCLUSION

Principle component regression (PCR) and partial least-square regression (PLS-1) models were successfully developed for determination of ACP and CZX in a standard mixture set (test set) which was not contributed in the calibration step. Similar accuracy was obtained from two

multivariate calibration methods. The same results were also performed when multivariate calibration models were applied to determine drugs in tablets. To evaluate the results obtained by multivariate calibration methods, a HPLC procedure was also used. The results obtained from PLS-1 and PCR models were not significant different with those obtained from HPLC method. This implies that the proposed PCR and PLS-1 models can comparable with HPLC method and could be applied very well for determination of ACP and CZX in tablets.

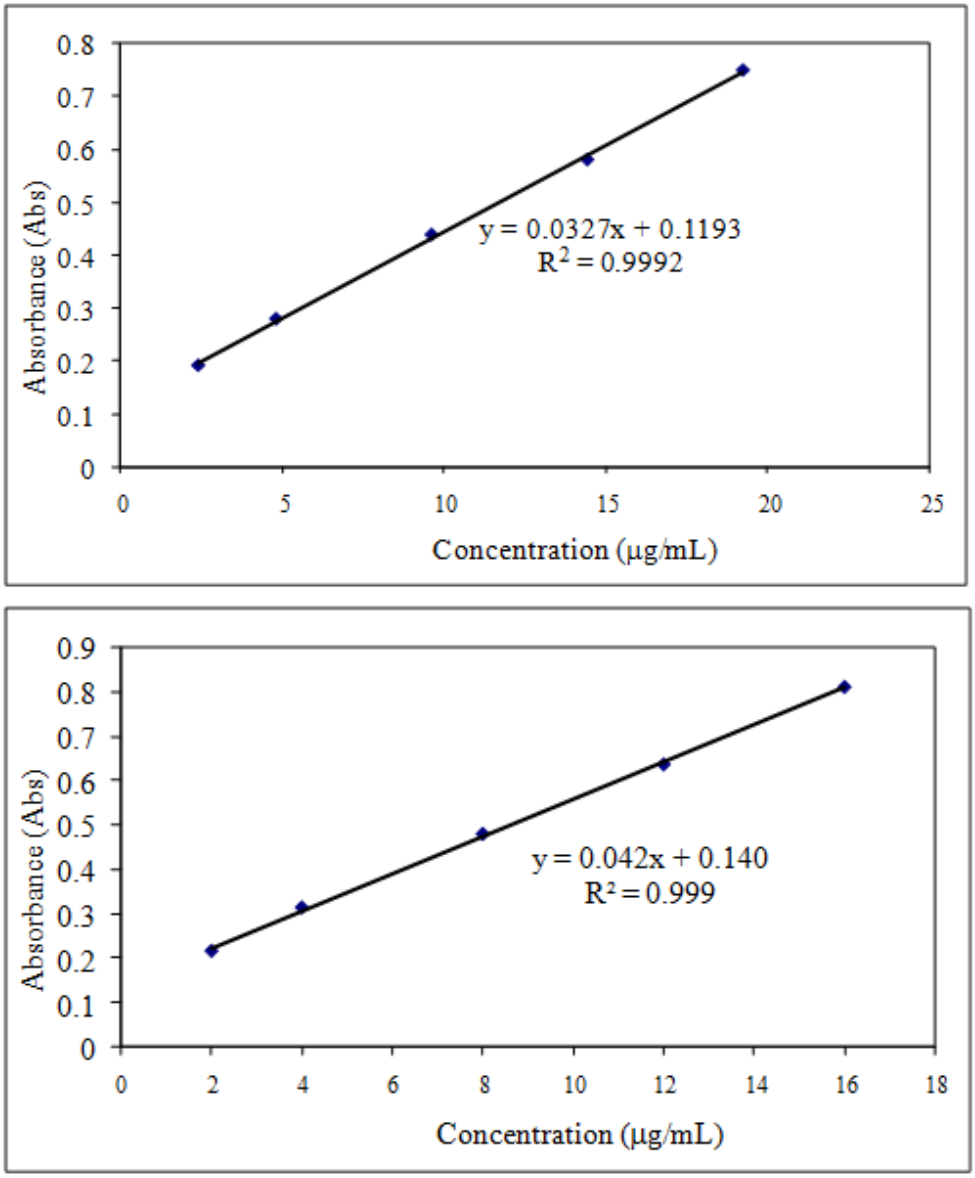


Figure 4. Linear dynamic range plots of ACP (4a) and CZX (4b)

Table 3. Statistic parameters of optimum PCR and PLS-1 models

Parameters	**PCR**	**PLS-1**
Acetaminophen		
Wavelength region (nm)	245-260	245-260
Number of factors	4	2
PRESS*	0.212	0.225
SEP**	0.139	0.143
r^2	0.9994	0.9994
Chlorzoxazone		
Wavelength region (nm)	200-240	200-240
Number of factors	4	4
PRESS*	0.584	0.601
SEP**	0.230	0.234
r^2	0.9977	0.9976

* PRESS = Prediction residual sum of squares
**SEP = Standard error of prediction

Table 4. Comparison of PCR and PLS-1 models for determination of ACP and CZX in test set

Test set number	**ACP (% recovery)**		**CZX (% recovery)**	
	PLS-1	**PCR**	**PLS-1**	**PCR**
1	104.3	103.0	96.4	96.8
2	103.3	103.3	99.9	100.0
3	99.4	98.7	98.5	98.6
4	100.5	100.5	99.2	99.2
5	100.7	100.7	100.7	100.7
6	99.0	99.0	106.5	106.5
7	100.0	102.0	100.0	100.6
8	101.8	103.6	101.4	101.4
9	99.0	99.5	100.8	100.8
Average	100.9	101.1	100.4	100.5
SD	1.9	1.9	2.7	2.6

Table 5. Determination results of ACP and CZX in tablets

Sample	% Labeled amount					
	PCR		PLS-1		HPLC	
	ACP	CZX	ACP	CZX	ACP	CZX
A1	89.7	99.4	90.3	99.5	90.6	98.4
A2	89.5	92.7	89.4	92.9	89.9	101.0
A3	90.7	94.9	90.8	95.0	86.8	96.1
A4	89.5	93.2	90.3	93.4	87.3	96.1
A5	89.9	93.1	90.6	93.2	90.2	97.7
Average	89.8	94.7	90.3	94.8	89.0	97.9
SD	0.5	2.7	0.6	2.7	1.7	2.0
B1	87.4	93.1	87.4	93.1	87.5	95.3
B2	90.1	95.5	90.0	95.5	88.5	96.2
B3	93.5	98.0	93.1	98.1	89.1	96.9
B4	88.1	92.7	88.0	92.9	88.2	95.8
B5	91.0	96.9	91.2	97.0	87.1	96.6
Average	90.0	95.2	90.0	95.3	88.1	96.2
SD	2.4	2.3	2.3	2.3	0.8	0.6

ACKNOWLEDGEMENTS

The authors would like to thank The Thailand Research Fund (MRG5280225) for financial support and the Department of Pharmaceutical Chemistry, Faculty of pharmacy, Mahidol University for research facilities.

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