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Research Article

HPLC and chemometrics-assisted spectroscopic methods used for determination of dissolution of paracetamol and orphenadrine citrate in a combination tablet

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ABSTRACT

Simultaneous determination of paracetamol and orphenadrine citrate by high-performance liquid chromatographic (HPLC) and chemometric-assisted spectroscopic methods are described. The HPLC method was based on RP C18 column (5 µm, 4.6 mm×150 mm) using monobasic ammonium phosphate, methanol, and acetonitrile (400:450:150v/v/v) as a mobile phase. The flow rate was set at 1.5 mL/min with column temperature at 40°C and UV detection at 215 nm. Paracetamol and orphenadrine citrate were separated within 7 mins by an isocratic elution. Good linearities were obtained in concentration ranges of 5-150 µg/mL for paracetamol and 0.8-12 µg/mL for orphenadrine citrate, with correlation coefficients (r)>0.99. Recovery of the analytical method was acceptable (102.8-104.8% for paracetamol and 92.4-102.3% for orphenadrine citrate). Relative standard deviations (RSDs) of repeatability and intermediate precision were less than 2.0%. Likewise, the resolution has been completed by using partial least square regression applying UV spectrum. The successive partial least squares regression (PLSR) methods were used with UV spectra data of 200-400 nm and 5 latent factors for paracetamol and orphenadrine citrate. Finally, the developed methods proved to be suitable to assay the dissolution samples of paracetamol and orphenadrine citrate in the combination tablet.

Keywords:
Paracetamol, Orphenadrine, HPLC-PDA, Partial least squares regression, Multicomponent analysis

1. INTRODUCTION

Paracetamol (4-acetamidophenol) is an effective analgesic and antipyretic for treatment of minor, non-inflammatory conditions[1]. Orphenadrine citrate ((RS)-(dimethyl-2-(2-methylbenz-hydroxy) ethyl) amine citrate) is employed as skeletal muscle relaxant[2]. Thus, tablets containing paracetamol (PAR) and orphenadrine citrate (OPC) show combined analgesic, antipyretic and skeletal muscle relaxing actions. The structures of PAR and OPC are displayed in Figure 1.

Many analytical methods have been reported on the estimation of paracetamol and orphenadrine citrate either separately or in combination with other drugs in pharmaceutical dosage forms or biological fluids[3-9]. However, only RP-HPLC[8], and spectrophotometric[10] methods have been described in the literature for the simultaneous determination of PAR and OPC in their combined formulations, despite the recognized commercial distribution of their tablets. Moreover, there is no official method for dissolution testing of their combined formulations. To assay the dissolution samples of drug products, a straightforward but broadly relevant analytical method is always preferred. Apparently, PAR and OPC fixed combination tablet considered to be an analytically challenging mixture, from the spectrophotometric viewpoint. This is ascribed not only to the large difference in ratios between PAR and ORP, which exceeds 1:12, respectively, but also the spectral overlap of both drugs. Consequently, analysis of PAR and OPC could not be performed concurrently by direct UV spectrophotometry without separation.

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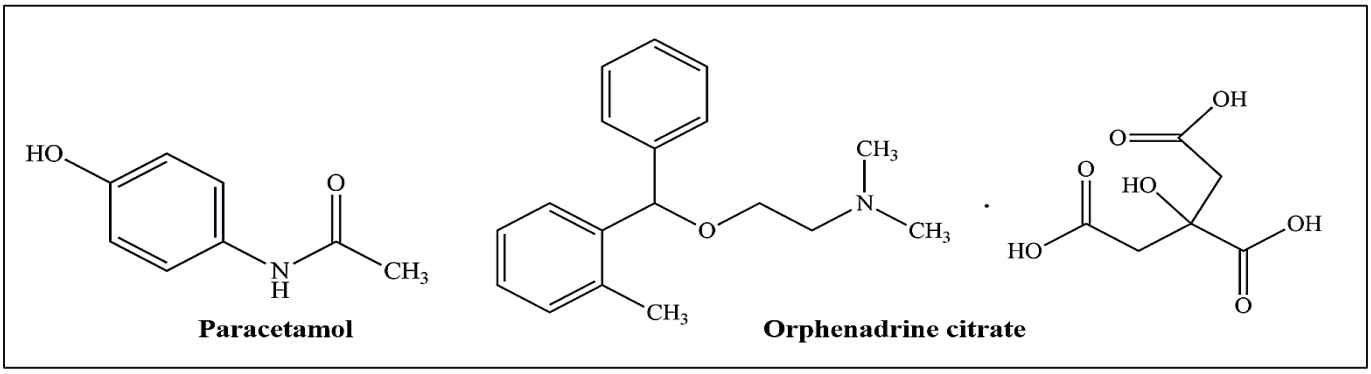

Figure 1. Chemical structure of Paracetamol and Orphenadrine citrate.

Application of chemometric approach with spectrophotometric data may overcome this restriction. Multivariate calibration is a chemometric method which has been utilized for determination of drugs in combination dosage forms including tablets[11-13].

In this study, chemometric-assisted spectrophotometry using partial least squares regression (PLSR) was developed to analyze the dissolution sample of PAR and OPC in combination tablet. In addition, a simple high-performance liquid chromatography (HPLC) was developed and validated to use as a reference method for chemometric method.

2. MATERIALS AND METHODS

2.1. Instruments and software

The analysis was carried out using HPLC system; a Shimadzu LC-10 system (Shimadzu, Kyoto, Japan) equipped with a model series LC-10 ADVP pump, SCL-10 AVP system controller, DGU-12A degasser, SIL-10ADVP auto injector, and a SPD-M20A diode array detector. A dual beam Shimadzu (Kyoto/Japan) UV-Vis. spectrophotometer, model 1650 UV-PC. The utilized software was UV-Probe personal spectroscopy software version 2.71 (SHIMADZU). Drugs' dissolution was tested using a ERWEKA DT 720 (Germany) equipped with standard USP type-II paddle.

2.2. Chemicals, reagents, and pharmaceutical formulation

All chemicals were of analytical-reagent grade. HPLC grade methanol and acetonitrile (Merk, Darmstadt, Germany) and deionized water were used for preparing mobile phase solutions. The standards for Paracetamol (PAR) and Orphenadrine citrate (OPC) were kindly supplied by Defense Pharmaceutical Factory, Bangkok, Thailand. The purity was found to be 100.0±0.1% and 100.1±0.2% for PAR and ORP, respectively, using the current compendial HPLC method of each drug[14,15]. ORPHETAMOL® tablets, manufactured by Defense Pharmaceutical Factory (DPF), Bangkok, Thailand, is labeled to contain paracetamol 450 mg and orphenadrine citrate 35 mg per tablet.

2.3. High-performance liquid chromatographic method

2.3.1. Chromatographic condition

The chromatographic separation was performed on a column C18, Hypersil GOLD®, 5 µm, 4.6x150 mm i.d., (Thermo, USA) with the column temperature maintained at 40°C. The mobile phase was obtained by mixing 0.05 M monobasic ammonium phosphate pH 7.9: methanol: acetonitrile (400:450:150 (v/v/v)), delivered at a flow rate of 1.5 mL/min and detected by ultraviolet at 215 nm.

2.3.2. Solutions

Standard stock solutions of PAR and OPC in mobile phase were separately prepared in concentration of 12.5 mg/mL and 1 mg/mL, respectively. One milliliter of each stock solution was transferred to a 50 mL volumetric flask and adjusted with mobile phase to obtain PAR and OPC in concentration of 250 µg/mL and 20 µg/mL, respectively. The working standard mixtures were prepared at concentration levels of 5-150 µg/mL and 0.2-12 µg/mL for PAR and OPC, respectively.

For placebo solution, 96 mg powdered placebo was weighed and introduced in a vessel of dissolution apparatus containing 900 mL of distilled water, thermostatically controlled at 37±0.5°C. A vessel content was agitated using a paddle at a rate of 50 rpm. for 60 min.

Sample solutions prepared for recovery study were in levels of 50% (50 µg/mL PAR, 4 µg/mL OPC), 100% (100 µg/mL PAR, 8 µg/mL OPC), 150% (150 µg/mL PAR, 12 µg/mL). Related amount of PAR, OPC each level with 96 mg placebo was weighed and placed in a vessel of dissolution apparatus containing 900 mL of distilled water, thermostatically controlled at 37± 0.5°C. A vessel content was agitated using a paddle at a rate of 50 rpm. for 60 min.

The placebo and sample solutions were withdrawn from the dissolution medium and filtered.

Two milliliters of each filtrate were transferred to 50-mL volumetric flask and adjust to volume by mobile phase. All solutions were passed through a polytetrafluoroethylene (PTFE) syringe filter prior to injection.

2.3.3. Validation of HPLC method

The proposed HPLC method was validated in term of specificity, linearity, range, accuracy, precision, and system suitability according to the International Council for Harmonization (ICH)[16]. For specificity, the chromatograms of mobile phase, distilled water, standard solution, and sample solution taken from dissolution study were compared. The linearity was assessed by analyzing the series of working standard mixtures in mobile phase. Six concentration levels in the range of 5-150 µg/mL and 0.2-12 µg/mL for PAR and OPC, respectively, were prepared. A regression equation of the calibration curve was calculated using least-square linear regression (correlation coefficient (r) ≥ 0.990). The accuracy was operated as described in sample solution preparation at levels of 50% (3 samples), 100% (3 samples) and 150% (3 samples). Recovered amount of PAR and OPC were calculated in relation to the added amount, whereas the acceptance criteria for recovery was 95-105%. In the precision study, repeatability and intermediate precision were carried out by analyzing 6 sample solution preparation at level of 100% on the same day (n=6) and 2 different analysts (n=12), respectively. The acceptance criterion for RSD was ≤ 2.0%. System suitability was determined from 5 replicate injections of the system suitability standard (40 µg/mL PAR and 8 µg/mL OPC) before sample analyses. The acceptance criteria were; number of theoretical plates (N) > 1500, tailing factor < 2 and %RSD ≤ 2.0 for peak area.

Table 1. Composition of calibration set samples.

Calibration sample	PAR (µg/mL)	OPC (µg/mL)	Calibration sample	PAR (µg/mL)	OPC (µg/mL)
1	39.1	14.8	25	19.5	7.4
2	57.7	14.3	26	28.9	7.1
3	49.8	2.8	27	24.9	1.4
4	48.5	27.2	28	24.2	13.6
5	46.4	9.3	29	23.2	4.6
6	54.1	9.4	30	27.0	4.7
7	55.6	25.5	31	27.8	12.8
8	45.6	25.6	32	22.8	12.8
9	49.6	15.9	33	22.5	7.8
10	47.5	15.7	34	24.8	8.0
11	48.6	15.0	35	23.8	7.8
12	48.6	0.0	36	24.3	7.5
13	49.1	0.0	37	24.3	0.0
14	9.2	14.9	38	24.6	0.0
15	60.6	27.5	39	4.5	7.3
16	61.9	28.4	40	31.0	14.2
17	59.7	28.5	41	29.8	14.2
18	61.9	28.8	42	24.7	7.0
19	49.9	14.0	43	24.9	6.9
20	49.4	13.8	44	19.1	0.0
21	38.8	0.0	45	29.8	2.7
22	38.3	0.0	46	29.5	2.8
23	39.0	0.0	47	29.5	2.7
24	38.7	0.0	48	29.3	2.7

Table 2. Composition of test set samples.

Test sample	PAR (µg/mL)	OPC (µg/mL)	Test sample	PAR (µg/mL)	OPC (µg/mL)
1	9.0	14.7	13	29.5	2.8
2	49.4	14.1	14	29.6	2.8
3	49.9	13.8	15	29.8	2.8
4	4.6	7.4	16	29.2	2.7
5	30.3	13.8	17	29.2	2.8
6	30.9	14.4	18	43.5	2.8
7	24.9	7.0	19	44.1	2.9
8	24.7	6.9	20	43.3	2.9
9	19.4	0.0	21	43.7	2.9
10	19.5	0.0	22	43.2	2.9
11	19.4	0.0	23	42.8	2.9
12	29.2	2.8	-	-	-

2.4. Chemometric method

2.4.1. Spectrophotometric condition

The calibration set and test set samples were recorded for their UV absorption data between 200-400 nm at 0.5 nm intervals using 1-cm quartz cells. The Unscrambler 9.6 (Camo, Norway) was employed for PLSR models construction.

Working standards solutions concentration of 250 µg/mL of PAR and 125 µg/mL OPC were prepared by accurately weighing the working standard by analytical balance and diluted to the desired concentration with deionized water. These working solutions were used to prepare calibration set and test set samples as shown in Table 1 and Table 2.

The models with the lowest relative standard error of prediction (RSEP) were selected as the optimum models.

RSEP was calculated by using the following equation.

$$PRSE(\%) = 100\sqrt{\frac{\sum_{i=1}^{m}(\boldsymbol{y_{pred}} - \boldsymbol{y_{ref}})^2}{m}}$$

Where m is the number of samples used, y_{ref} is the true value and y_{pred} is the predicted value of a test set sample.

2.4.2. Validation of chemometric method

The optimum PLSR models were internal validated by cross-validation and external validated by determination of test set samples those were not contributed to models construction and compared with HPLC results. The comparison was expressed as the correlation plot of the results from PLSR model (y-axis) and HPLC method (x-axis). The correlation coefficient closes to 1.0 indicating the agreement of the results of two methods and imply to the accuracy of chemometric model.

2.5. Assay of dissolution solutions

An eight-vessel dissolution apparatus containing 900 mL of distilled water, thermostatically controlled at 37±0.5°C was used, the tablet was introduced into the paddle using a rate of 50 rpm. Samples were withdrawn from the dissolution medium after 60 min and filtered. Two milliliters of the filtrate were transferred to 50-mL volumetric flask and adjust to volume by either mobile phase for HPLC method or distilled water for chemometric method.

3. RESULTS AND DISCUSSION

3.1. Development of HPLC method

The method optimized for PAR and OPC was primarily performed using various mobile phases for improved peak shape and separation. The mobile phase initially investigated comprised of water adjusted with phosphoric acid to pH 2.6 and acetonitrile (500:500), sodium dihydrogen phosphate pH 7.9 and acetonitrile (350:650) and monobasic ammonium phosphate pH of 3.2 and acetonitrile (400:600). In most of the mobile phases, the separation was inadequate and OPC was not detected. The best separation was achieved using 0.05 M monobasic ammonium phosphate pH 7.9, methanol, and acetonitrile (400:450:150). Two different C18 columns (Hypersil® 5 µm, 4.6 mm×250 mm and Hypersil GOLD®, 5 µm, 4.6 mm×150 mm) were tested as stationary phases for better symmetry of OPC peak. The Hypersil GOLD® C18 column exhibited better peak shape (tailing factor < 2.0) and shorter analysis time. The mobile phase was used as a better diluting solvent in this study after optimization. The flow rate (1 mL/min and 1.5 mL/min), wavelength (215 nm and 220 nm), injection volume (10 µL and 20 µL) was examined and adjustments were made appropriately. The optimal flow rate, wavelength, and injection volume for the method was 1.5 mL/min, 215 nm, and 10 µL, respectively. The optimal conditions were designated based on better separation parameters of PAR and OPC peaks. Figure 2C and Figure 2D illustrate the HPLC chromatograms of standard and sample solution of PAR and OPC, respectively.

3.2. Validation of HPLC method

The chromatogram of dissolution sample exhibited the good separation of PAR and OPC from other peaks in the sample with tailing factor less than 2.0. No major peak other than PAR and OPC in dissolution medium (Figure 2A) and mobile phase (Figure 2B) was observed, there was no interference between excipients (Figure 2C and Figure 2D). Moreover, the UV spectrum from 200-400 nm of the peak in the sample with the retention times matching to the peaks of PAR (RT of 1.4 min) and OPC (RT of 6.3 min) standards were similar with peak purity index more than 0.95. The calibration curves of PAR and OPC were linear in the ranges of 5-150 µg/mL and 0.2-12 µg/mL, respectively. The correlation coefficients (r) of the linear equations were more than 0.999, thus confirming the linearity of the methods. The recoveries of PAR and OPC were 102.8-104.7% and 92.4-102.3% respectively, indicated a satisfactory accuracy. For repeatability, the %RSD values of PAR and OPC ranged from and 0.78-1.35% and 0.82-1.14%, respectively while the %RSD values of the intermediate precision were 0.88 and 0.97%, respectively. The proposed HPLC method showed acceptable validation and system suitability parameters as shown in Table 3.

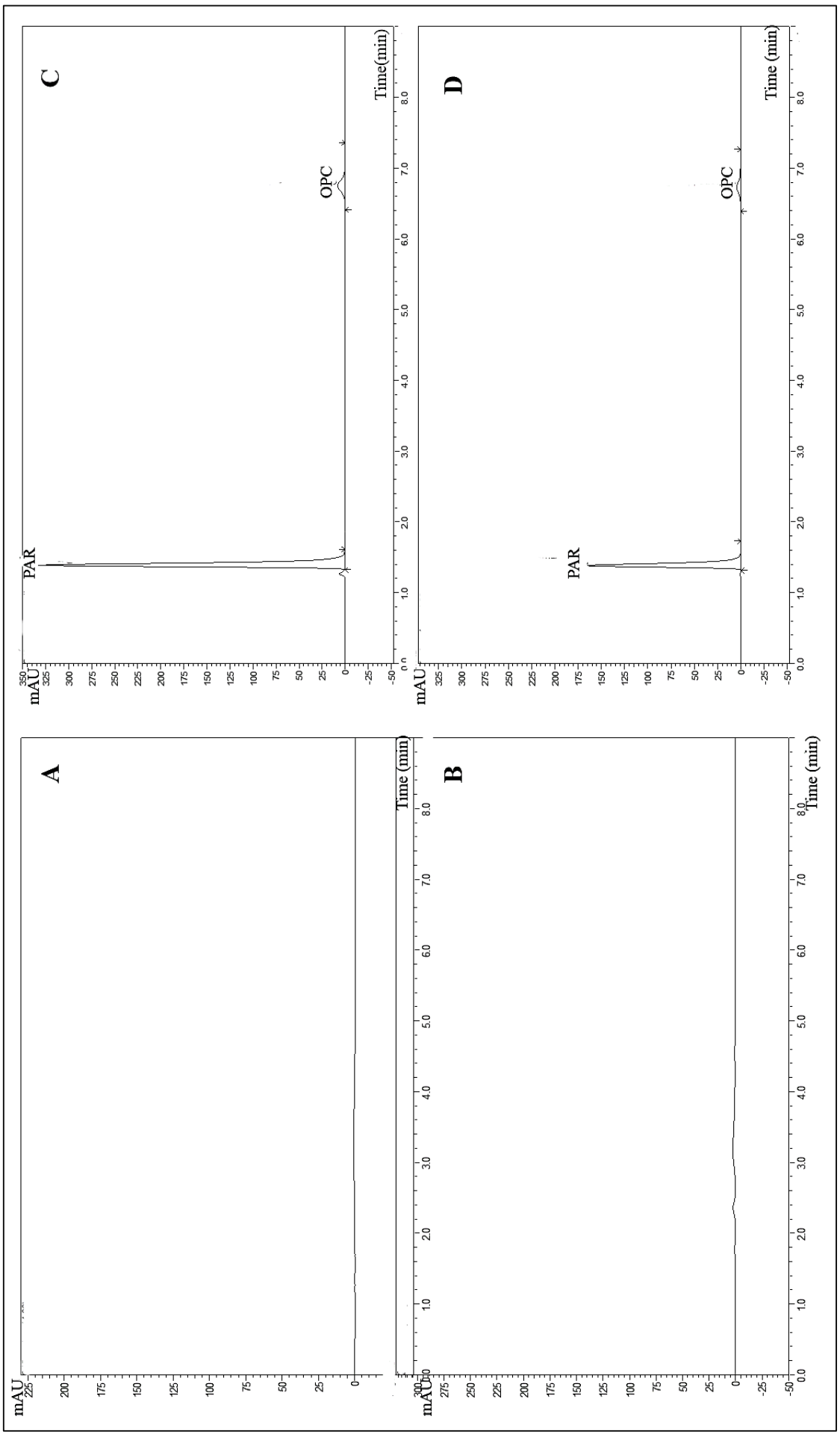

Figure 2. HPLC chromatograms of distilled water (A), mobile phase (B), standard mixture as 40µg/mL of PAR and 8µg/mL of OPC (C), and dissolution sample (D).

Table 3. Method validation data of PAR and OPC by the optimized HPLC method.

Validation parameter		Acceptance criteria	PAR	OPC
Linearity		r ≥0.99	y =12,614x+3,586.5 r =0.9999	y =12,684x-1,357.1 r =0.9997
Range			5-150 µg/mL	0.2-12 µg/mL
Accuracy (%R)		92-105%	102.8-104.7%	93.4-102.3%
Repeatability (%RSD, n=6)		2.0%	0.78-1.35	0.82-1.14
Intermediate precision (%RSD, n=12)		2.0%	0.89	0.97
System suitability	Plates	>1500	>2338	>6826
	Tailing	<2.0	<1.6	<1.1
	%RSD	<2.0	0.74	0.33

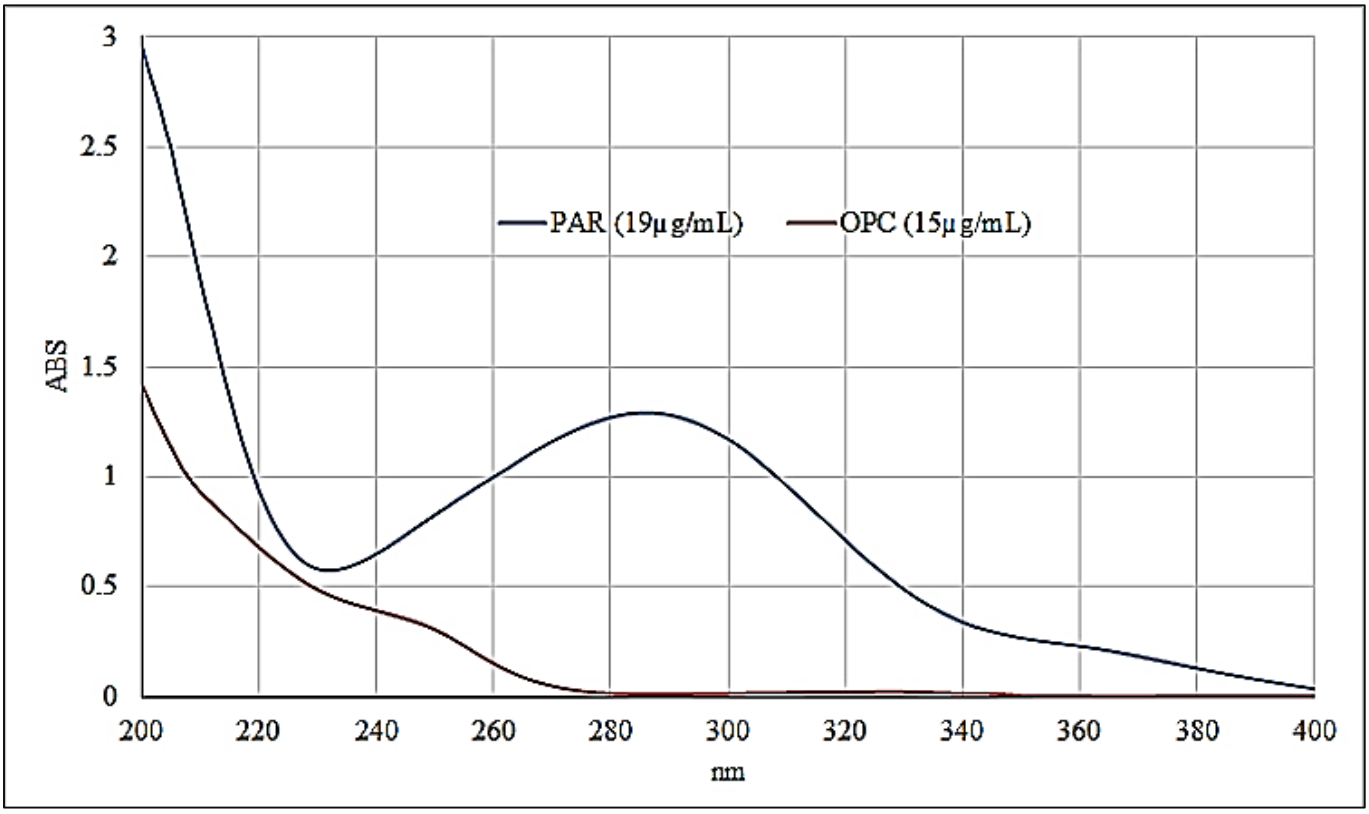

Figure 3. UV spectra of PAR (19 µg/mL) and OPC (15 µg/mL).

3.3. Development of Chemometric method

UV spectrophotometry is generally allowed for quantitative determination of an active pharmaceutical ingredient with high purity. Direct determination of combination drugs by UV spectrophotometer is usually limited from the overlapping of their UV spectra. Several efforts were tried to overcome this limitation including first derivative and higher order UV spectra, mean centering of ratio spectra. The main problem of this study is that the concentration of OPC in tablet is very low, compares with PAR. The tablet formula in Thailand contains 35 mg of OPC and 450 mg of PAR per tablet. As shown in Figure 3, UV spectra of PAR and OPC are completely overlapped in UV region. And unfortunately, the molar absorptivity of PAR and OPC are quite different. As seen in Figure 3, UV absorbance of 19 µg/mL of PAR is dominated and covered absorbance signal of 15 µg/mL of OPC. Therefore, simultaneous quantitative determination of OPC and PAR with indirect UV spectrophotometric techniques as described above were not success. In this study, partial least square regression (PLSR), a widely used chemometric technique, was tried for determination of OPC and PAR in dissolution samples. The success PLSR model for determination of PAR and OPC were performed by using UV absorption data between 200-400 nm with 5 latent factors. The parameters of OPC and PAR optimum PLSR models are showed in Table 4.

3.4. Validation of chemometric method

The internal validation of PLSR models were cross-validation. The results of cross-validation of the successive PLSR models were displayed as the plot between actual concentrations and predicted concen-

trations of the samples in the calibration set (Figure 4). The slope and correlation coefficient (R^2) close to 1.0 indicating the agreement of actual and predicted concentrations and assure the future application. The developed PLSR models were external validated by quantitation of test set samples. The determination results were expressed by the plot between actual concentrations and predicted concentrations of the samples in the test set (Figure 5). RSEP of the optimum models were calculated, the minimum RSEP indicating the accuracy and precision of the model. As shown in Figure 4 and Figure 5, the slope of the plots was around 1 (0.9978 for PAR and 0.9980 for OPC) and the R^2 values were higher than 0.99. The RSEP values, as shown in Table 5, were less than 6%. These results indicated that the PLSR models for OPC and PAR were accurate and suitable for intend purpose.

3.4.1. Assay of dissolution solutions

Dissolution solutions of six combined tablets performing under USP condition of OPC in the combination tablets[17] were taken and assayed by the developed HPLC method and PLSR models. The assay results as quantity percent (%Q) of HPLC and PLSR models were compared using *t*-test at 95% confidence level. There was no significant difference (*P*-values >0.05) between the results as indicated in Table 5.

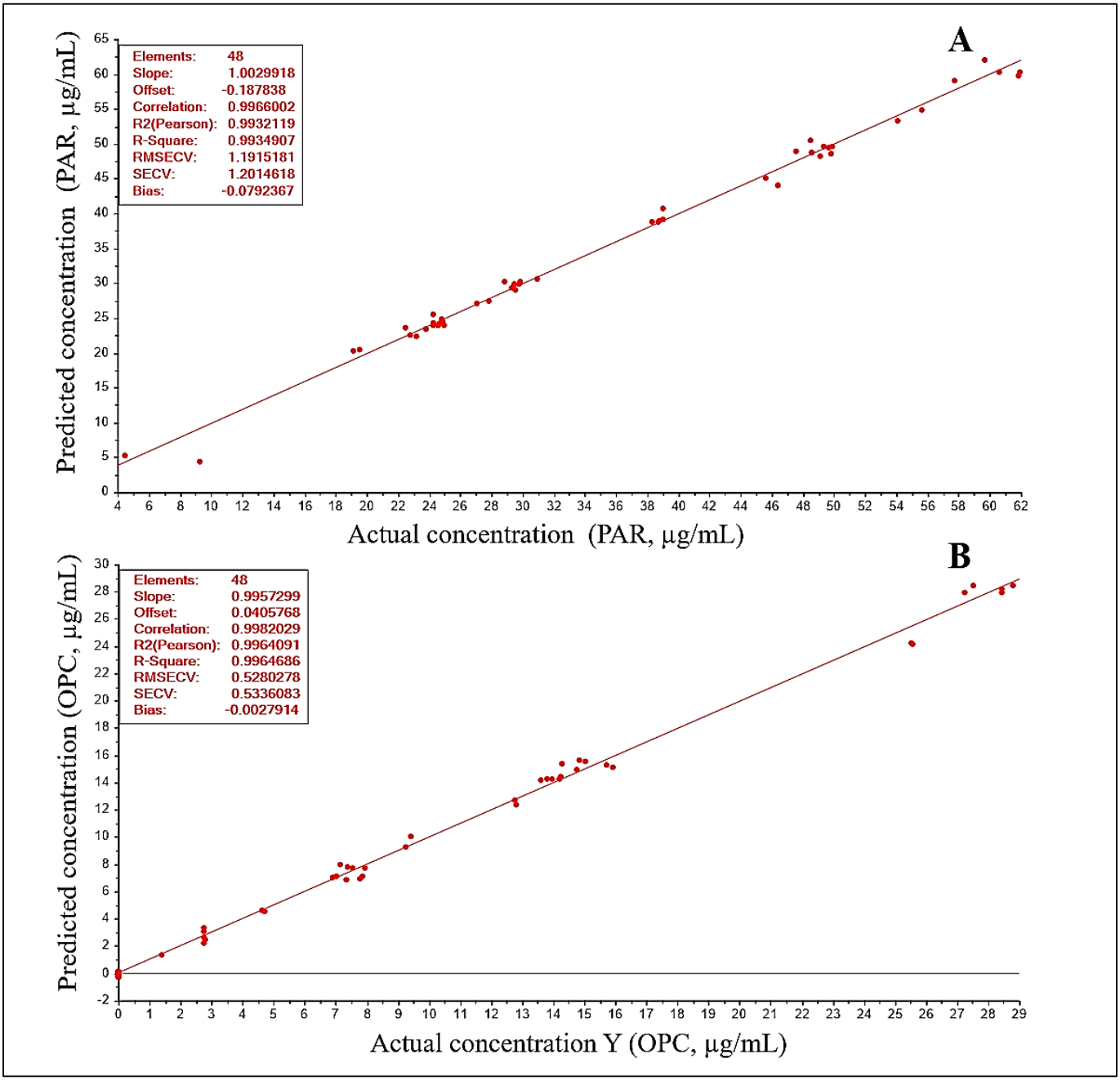

Figure 4. The cross-validation plots of (A) PAR and (B) OPC.

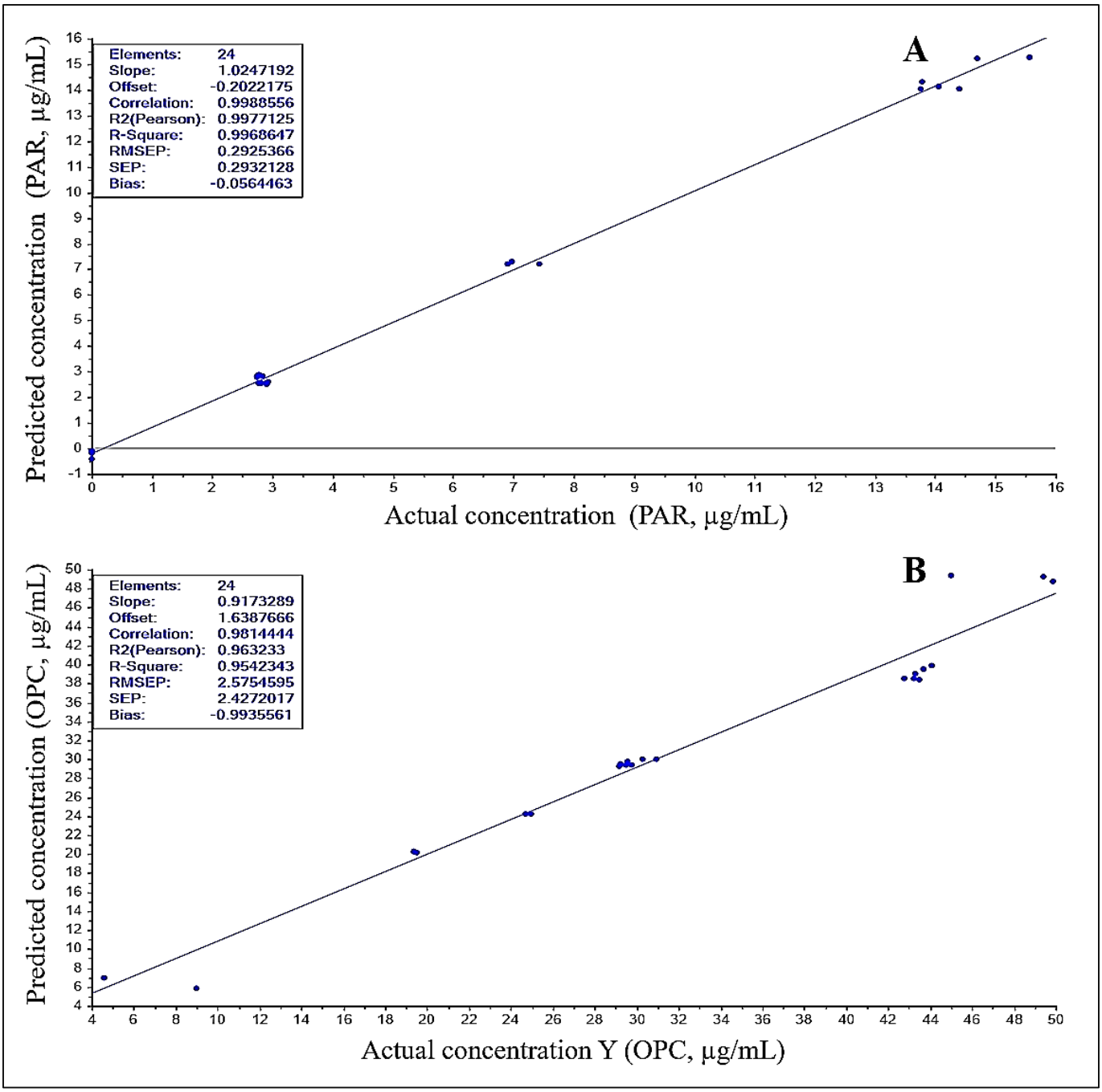

Figure 5. The correlation plots between actual and predicted concentrations of test set samples for (A) PAR and (B) OPC.

Table 4. The parameters for PAR and OPC of optimum PLSR models.

Model Parameters	PAR	OPC
Wavelength (nm)	200-400	200-400
Latent factors	5	5
Calibration	-	-
Slope	0.9978	0.9980
Offset	0.0796	0.0201
R^2	0.9978	0.9980
Cross-validation	-	-
Slope	0.9940	0.9957
Offset	0.2163	0.0406
R^2	0.9960	0.9965
RSEP (%)	5.96	3.98

Table 5. The comparison of %Q obtained from HPLC and PLSR methods.

Tablet number	PAR		OPC	
	HPLC	PLSR	HPLC	PLSR
1	96.7	96.0	80.4	81.6
2	98.0	99.8	83.3	83.1
3	96.1	97.6	81.5	82.8
4	97.0	98.8	81.6	81.2
5	95.9	96.4	82.6	82.5
6	95.1	96.4	82.5	82.3
P-values	0.21		0.63	

4. CONCLUSIONS

The HPLC and chemometric-assisted spectrophotometric methods have been proposed and successfully applied for the simultaneous determination of PAR and OPC. The dissolution results obtained by chemometric method were found to be in good agreement with that of HPLC method with the percentage release of all two drugs were above 80% in 60 min. The HPLC method is more specific than the chemometric-assisted spectrophotometric method, but it requires costly equipment and materials, for example, columns and HPLC grade solvents. Chemometric method is less expensive and does not need complicated instrumentation and any separation steps. The proposed HPLC and PLSR methods were found to be suitable and can be effectively used to assay the dissolution samples of PAR and OPC in fixed dose combination tablet.

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Conflict of interest

The authors wish to confirm that there are no known conflicts of interest related with this publication.

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Ethics approval

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