Quantitative Determination of Clindamycin Phosphate in Gel Preparation Using PLSR Model

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Quantitative determination of clindamycin by UV spectrophotometry is limited due to the lack of any UV chromophore of clindamycin structure. In this study, UV spectrophotometry employing partial least square regression (PLSR) model was developed with respect to high-performance liquid chromatography (HPLC) as the reference method for the quantitative determination of clindamycin phosphate in gel preparation. The successful PLSR model used UV spectral data in the region of 190-400 nm without data preprocessing. The leave-one-out cross-validation was utilized for model construction. The latent factor 2 was used in the final model with the R^2 model of 0.9972, root mean square error of calibration (RMSEC), and root mean square error of cross-validation (RMSECV) of 0.0044 and 0.0048, respectively. Model ability was approved by quantitative determination of 20 validation samples that were not contributed in the model-building step. The accuracy of determination results expressed in terms of root mean square error of prediction (RMSEP), the relative standard error of prediction (RSEP%), and bias were 0.0072, 0.0447%, and -0.0025, respectively. This study demonstrated that PLSR-assisted UV spectrophotometry could be used as an alternative method for the quantitative determination of non-UV chromophore active pharmaceutical ingredients in the pharmaceutical dosage form.

INTRODUCTION

Clindamycin, a lincosamide antibiotic, was developed in 1966 by chemically modifying the naturally occurring lincomycin. Clindamycin provides excellent activity against both Gram-positive cocci and Gram-positive or negative anaerobes, it has been used for the treatment of head and neck, respiratory, bone and soft tissue, abdominal, and pelvic infections [1,2]. Since clindamycin lacks UV chromophore (Fig. 1), UV spectra of clindamycin present the absorption

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band between 190-230 nm and unfortunately, the excipients in gel preparation can be absorbed in the same wavelength region. The assay of clindamycin in gel described in the United State Pharmacopeia (USP) 2022 is liquid chromatograph, the condition consists of L7 (4.6-mm × 25-cm) column, a mobile phase mixture of 0.1M monobasic potassium phosphate, pH 2.5 and acetonitrile (77.5/22.5: v/v), and UV detector at 210 nm [3]. Besides the USP assay method, there were reports of quantitative determination of clindamycin in the combination of other active pharmaceutical ingredients and plasma, most of them were employed with high-performance liquid chromatography
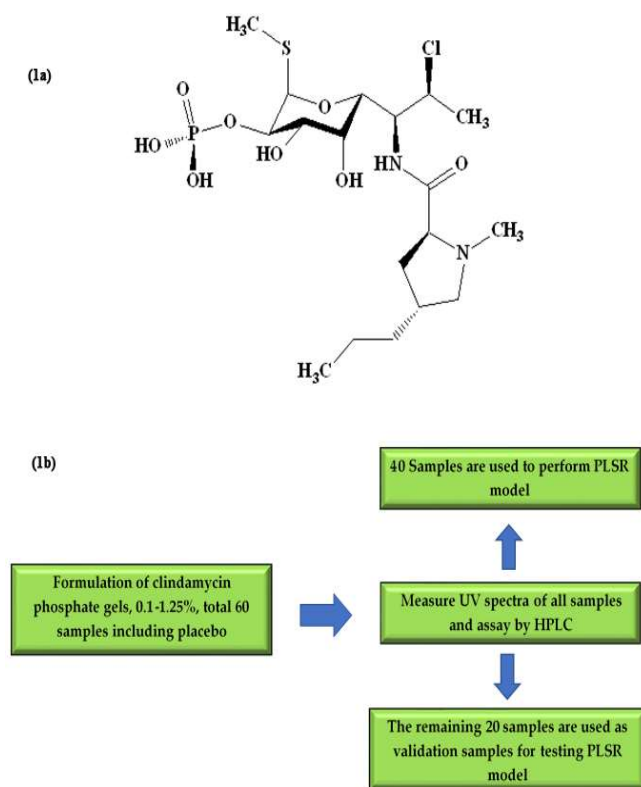
Fig. 1. (a) Chemical structure of clindamycin phosphate, (b) Schematic diagram of samples arrangement.

connected with UV, photodiode array, and mass spectroscopy [4-9]. Quantitative determination of clindamycin content in gel preparation by UV spectrophotometry without chromatographic separation is limited due to lacking UV chromophore of clindamycin structure and suffering from gel base interferents as mentioned above. This is our challenge to perform this study with the aid of a chemometrics approach.

From the first invention of chemometrics, the chemical discipline that uses mathematical, statistical, and other methods employing formal logic to design or select optimal measurement procedures and experiments, and to provide maximum relevant chemical information by analyzing chemical data, [10,11], chemometrics has played the important role in multidisciplinary of sciences [12]. Quantitative determination of an interested substance in a mixture or complex sample without chromatographic separation is the most widely used application of chemometrics in the pharmaceutical analysis [13-18].

Multivariate analysis method namely partial least square regression (PLSR) is mostly employed for this application [18]. In addition, the guideline for the validation of multivariate analysis procedure has been developing [19]. The aim of this work is the development of a PLSR-assisted UV spectrophotometric method for the quantitative determination of clindamycin in gel preparation and compared it with HPLC which was modified from the USP method.

MATERIALS AND METHOD

Materials

Standard clindamycin phosphate was purchased from Supelco (Merck, Thailand). Working standard clindamycin phosphate was a generous gift from Siam Bheasach Co., Ltd. (Bangkok, Thailand). Absolute ethanol (AR grade) from PT. Murni Dharma Karya (Jakarta, Indonesia) was used for gel extraction. Acetonitrile, HPLC grade, was from Supelco (Merck, Thailand). Propylene glycol, carbomer (Carbopol® 941), carboxymethyl cellulose (CMC), and ethylenediamine tetraacetic acid (EDTA) were purchased from S. Tong Chemicals Co., Ltd. (Nonthaburi, Thailand). Ethanol (95% v/v) was supplied by Liquor Distillery Organization Excise Department (Chachoengsao, Thailand). Sodium hydroxide, from which a 10% w/v solution was prepared and used as a neutralizing agent, was purchased from CARLO ERBA Reagents S.A.S. (Val de Reuil, France). Purified water was used as a solvent for gel formulation.

Instruments

UV spectral data were corrected from UV 1900 (Shimadzu, Kyoto, Japan), and the spectral raw data were transferred to an Excel file for further PLSR modeling by the Unscrambler program (AspenTech, MA, USA). The Prominence 20AD of Shimadzu (Bara Scientific, Thailand) was used throughout the study. Another HPLC instrument for precision study in method validation was SpectraSystem (Thermo Separation, Amani, Thailand).

Gel Preparation

The composition of the analyzed gel base is given in Table 1. Carboxymethyl cellulose was weighed and dispersed into a beaker containing purified water. After

Table 1. Composition of Gel Base

Ingredient	Amount (%w/w)
Carbopol 941	2.0 g
Carboxymethyl cellulose	0.5 g
Propylene glycol	15.0 g
Ethylenediamine tetraacetic acid	0.1 g
95% v/v Ethanol	20.0 g
10% w/v Sodium hydroxide	q.s. to pH 5.0-5.5
Purified water	q.s. to 100.0 g

complete swelling, ethylenediamine tetraacetic acid, Carbopol® 941, propylene glycol, and ethanol were added and mixed well for 5 min. The pH of the formulation was then adjusted to pH 5.0-5.5 using 10% w/v sodium hydroxide solution and stirred slowly until a clear and transparent gel was obtained.

Formulation and Characterization of Gel Preparations

Clindamycin phosphate was added and mixed with a gel base to the expected final concentrations of clindamycin gel between 0.1-1.25% w/w. The physical appearance of formulations, pH, and viscosity were evaluated. pH was measured at 30.0 ± 0.5 °C using a pH meter (FiveEasy Plus, Mettler Toledo, Greifensee, Switzerland). Viscosity measurements were performed using a rotational rheometer (cone and plate model C35/2° TiL-222-1871, HAAKE™ RotoVisco™, Thermo Fisher Scientific, Karlsruhe,

Germany) at a shear rate of 5 s⁻¹ and a temperature of 30.0 ± 0.5 °C. The physical appearance of formulations, pH, and viscosity are shown in Table 2.

Sample Preparation

Five grams of gel was accurately weighed, dissolved, and adjusted to 50 ml with 80% ethanol. The mixture was mechanically shaken for 30 min and then centrifuged at 4500 rpm for 15 min. Two milliliters of supernatant were transferred to a 10-ml volumetric flask and adjusted to volume with the mobile phase. This sample solution was measured by UV spectrum and quantitated by HPLC. For HPLC determination, the solution was filtered through a 0.45 μm nylon syringe filter membrane before injection into the HPLC instrument.

HPLC Method Validation

The HPLC condition consists of an L7 (4.6-mm × 15-cm) column, a mobile phase mixture of 0.01 M monobasic potassium phosphate, pH 2.5 and acetonitrile (80/20: v/v), and UV detector at 210 nm. The method was validated for linearity, accuracy, precision, and specificity to confirm suitability for the intended purpose.

Development of PLSR Method

Five gel preparations and placebo gel were divided into 10 sub-samples for each to obtain a total of 60 sub-samples (Fig. 1). The 60 sub-samples were separately determined the actual clindamycin phosphate contents by using the HPLC method described above. All sub-samples were corrected for their UV spectra between 200-400 nm by using 80% ethanol

Table 2. Characterization of Gel Preparations

Formulation	Characterization		
	Appearance	pH*	Viscosity* (cP)
Gel base	Clear gel	5.1 ± 0.0	$54,314 \pm 689$
0.10% w/w Clindamycin gel	Clear gel	5.1 ± 0.1	$53,890 \pm 1,054$
0.25% w/w Clindamycin gel	Clear gel	5.2 ± 0.1	$56,675 \pm 807$
0.50% w/w Clindamycin gel	Clear gel	5.2 ± 0.0	$54,976 \pm 729$
0.75% w/w Clindamycin gel	Clear gel	5.2 ± 0.2	$55,602 \pm 1,051$
1.25% w/w Clindamycin gel	Clear gel	5.2 ± 0.1	$53,946 \pm 1,253$

*Data are expressed as mean $\pm$ standard deviation (n = 3).

as blank. From 60 sub-samples, 40 sub-samples (including 10 placebo sub-samples) were used as calibration samples set in PLSR model construction, and the remaining 20 sub-samples were used as validation samples set.

Various PLSR models were performed and validated from the UV spectra of 60 sub-samples as described above with respect to the actual values from the HPLC method. The optimum PLSR model was selected and evaluated from the highest R^2 of the model and agreement between the root mean square error of calibration (RMSEC) and root mean square error of cross-validation (RMSECV).

Then, the optimum PLSR model was validated by the determination of clindamycin phosphate in the validation samples set. The determination results from the PLSR models were compared with the reference HPLC values by scatter plot. The R^2 Pearson of correlation plot and the residual plot were represented for the linearity of the PLSR model. Model error parameters such as root mean square error of prediction (RMSEP), the relative standard error of prediction (RSEP%), and bias were expressed for model accuracy and precision. RMSEP, RSEP%, and bias were calculated as Eqs. ((1)- (3)).

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{n}(y_i - y_{i,ref})^2}{n}} \quad (1)$$

$$RSEP\ (\%) = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i)^2}} \times 100 \quad (2)$$

$$Bias = \frac{1}{n}\sum_{i=1}^{n}(y_i - y_{i,ref}) \quad (3)$$

RESULTS AND DISCUSSION

HPLC Method Validation

HPLC determination of clindamycin phosphate was first attempted by using the condition that existed in USP 2022. However, there was some interferent signal presented close to the clindamycin phosphate peak with unappreciated resolution. Therefore, the mobile phase ratio was adjusted to use acetonitrile 20% and the concentration of monobasic potassium phosphate was reduced to 0.01M to avoid precipitation with acetonitrile mixing. The modified HPLC condition consists of an L7 (4.6-mm × 15-cm) column, a mobile phase mixture of 0.01 M monobasic potassium

phosphate, pH 2.5 and acetonitrile (80/20: v/v), and UV detector at 210 nm. Because the dimension of the column used in this study was beyond the allowed limit for L/dp of USP guideline [20], the modified condition was validated in terms of linearity, accuracy, precision, and specificity. All method validation results were accepted with the R^2 higher than 0.99 for the concentration range of 0.1-0.5 mg ml^{-1}. Accuracy was displayed in terms of percent recovery of standard addition at three concentrations (0.1, 0.3, and 0.5 mg ml^{-1}) and three replicates, % recovery ranged from 98.2-101.8%. Repeatability, expressed in terms of % RSD of 3 replicated determinations on the same day, ranged from 2.38-2.69%. Intermediate precision was performed by comparing repeatability results from two instruments, the %RSD of the two instruments was 0.23-2.69%. The specificity of the method was illustrated by comparing the chromatograms of placebo gel, standard and standard addition at 0.3 mg ml^{-1}. The compared relevant chromatograms show that the retention time of clindamycin phosphate in standard addition solution agrees with the retention time of clindamycin phosphate in standard solution. In addition, there was no interferent peak from the placebo present at the same retention time as the clindamycin peak. The HPLC method validation results are summarized in Table 3.

Development of PLSR Method

As seen from Fig. 1, clindamycin phosphate lacks UV chromophore and UV spectra of clindamycin phosphate gels present the absorption band at 190-230 nm which is the same region as placebo gel preparation (Fig. 2a). Therefore, quantitative determination of clindamycin phosphate in the gel was attempted with the aid of chemometrics-assisted UV spectrophotometry. The UV spectral data of 40 sub-samples were subjected to the Unscrambler program for PLSR modeling by using assay values from the developed and validated HPLC method as the reference concentrations. It was found that data pretreatment was not necessary for UV spectral data of clindamycin gel, the PCA plot of unpretreated data showed the samples grouping with concentrations (Fig. 3). Some PLSR models were obtained from the original data with various wavelength intervals. Finally, the model constructed from wavelength interval 200-400 nm with 2 latent factors, R^2 model of 0.9972, RMSEC of 0.0044, and
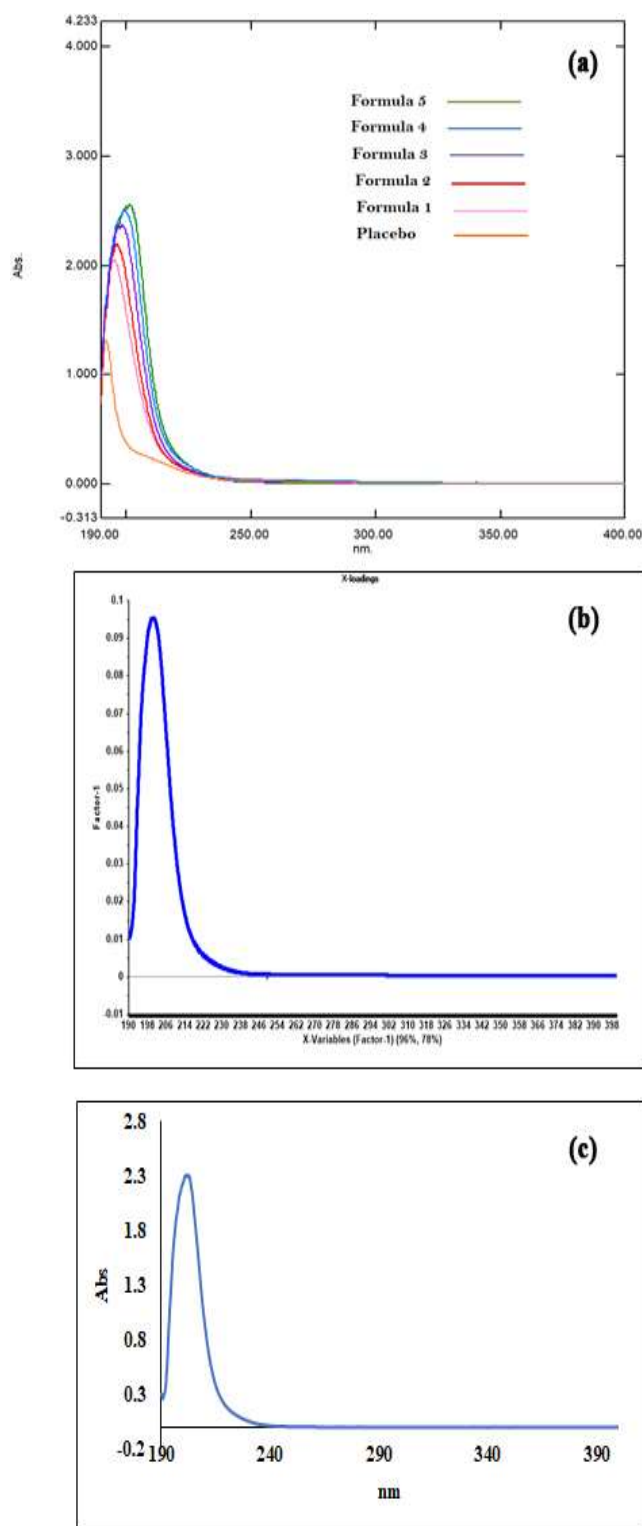
Fig. 2. (a) UV spectra of gel preparations, (b) Loading plot of final PLSR model, (c) UV spectrum of clindamycin phosphate.

RMSECV of 0.0048 were found to be the best model and further validated by determination of 20 sub-samples in the validation set. The concentrations of clindamycin in the validation set samples from PLSR and HPLC methods (Table 4) were statistically compared. The determination results obtained from the best PLSR model were not significantly different from the reference values obtained from the HPLC method at a 95% confidence interval with a p-value > 0.05.

The correlation plot between predicted and reference concentration shows R^2 Pearson of 0.9939, RMSEP of 0.0072, bias of -0.0025 (Fig. 4), and RSEP% of 0.0447, these results indicated linearity, accuracy, and precision of PLSR model for determination of clindamycin phosphate gel. The x-loading plot (Fig. 2b indicating the relevant variables used in the model show a consistent pattern with the UV spectrum of clindamycin phosphate standard (Fig. 2c).

In addition, good agreement between PLSR-assisted UV spectrophotometry and HPLC determination results were presented as Bland-Altman plots in Fig. 5 [21,22]. Almost the data points fall into ± 2SD of the mean difference. As shown in Table 5, although negative bias was found for the mean difference (-0.003), this was not significant since the lines of equality (Difference = 0.000) were with in the confidence interval of the mean difference (-0.006 - 0.001).

CONCLUSION

In summary, the application of PLSR for the quantitative determination of non-UV chromophore active pharmaceutical ingredients was successfully demonstrated in this study. Although absorption of excipients in gel preparation presented at the same UV region of the clindamycin band, PLSR could show the quantitative determination ability for clindamycin at very low concentrations without interfering with other excipients. However, sample preparation was required because UV measurement and HPLC determination are not allowed with unclear solutions. But this study demonstrated that the chemometrics-assisted UV spectrophotometric method could replace the HPLC instrument in the quantitative determination step.

Table 3. Method Validation Results of HPLC Method

| Method validation parameters	Results
Linearity (Equation, R^2)	
Day 1	y = 3566x + 245, R^2 = 0.9988
Day 2	y = 3517x + 66, R^2 = 0.9943
Day 3	y = 3379x + 6, R^2 = 0.9999
Accuracy	
-% Recovery	98.24-101.79
Precision	
-Repeatability (%RSD, n = 3)	2.38-2.69
-Intermediate precision (%RSD, n =3)	0.23-2.69
Specificity	
Retention time of clindamycin phosphate peak in standard spiked sample was agree with standard chromatogram	Complied
There was not excipient peak presents at the same retention time as clindamycin peak	Complied
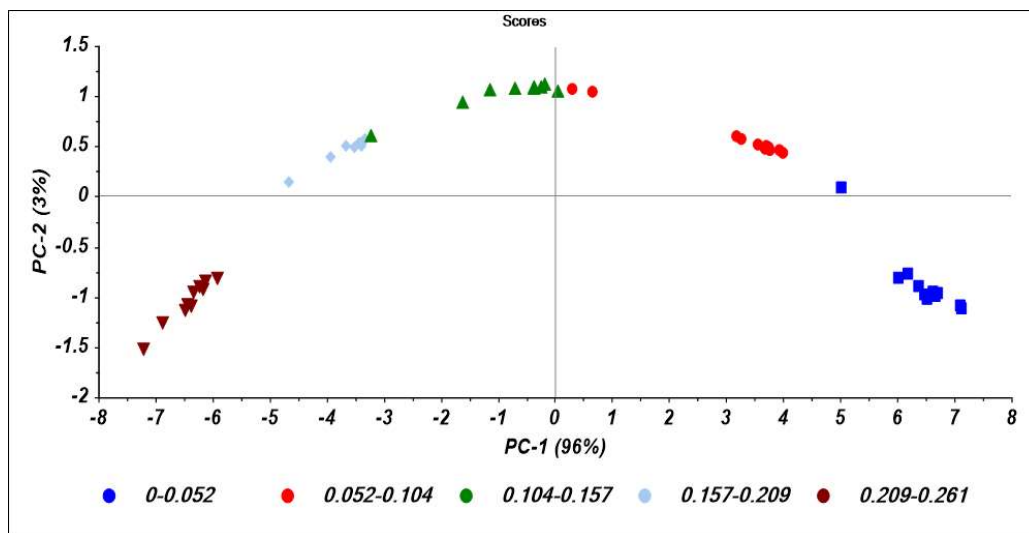
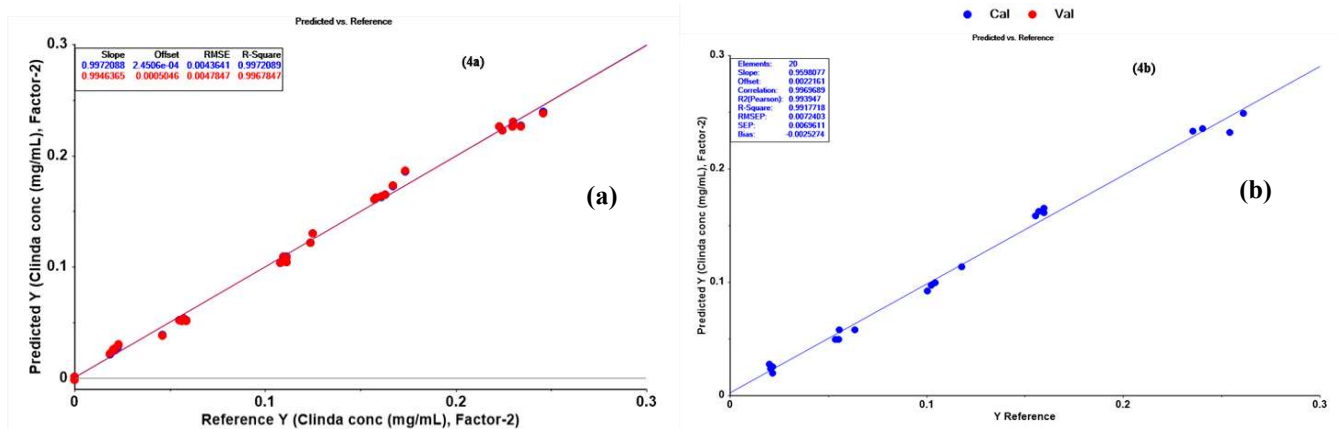**Fig. 3.** PCA plot of unpretreated spectral data shows the samples grouping with concentrations.

Fig. 4. (a) Plots of the PLSR model calibration and cross-validation (b) correlation plot of model prediction *versus* reference values.

Table 4. Quantitative Determination Results of Validation Set Samples and Statistical Comparison

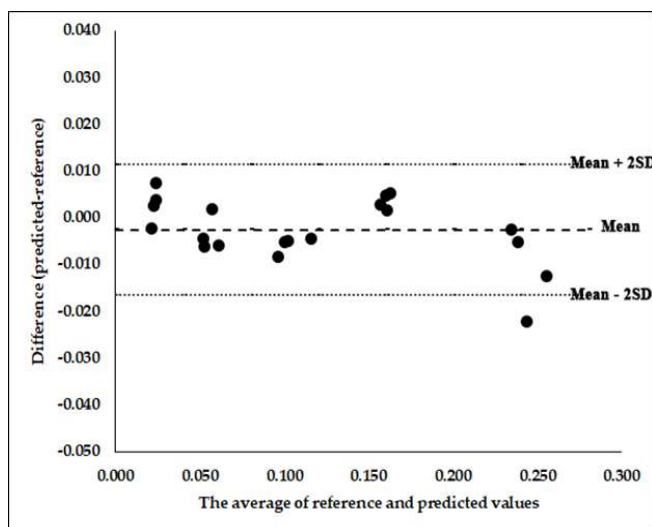| Validation samples	PLSR (mg ml^{-1})	HPLC (mg ml^{-1})
TF16	0.028	0.020
TF17	0.026	0.022
TF18	0.020	0.022
TF19	0.024	0.021
TF26	0.049	0.054
TF27	0.058	0.056
TF28	0.049	0.055
TF29	0.058	0.064
TF36	0.092	0.100
TF37	0.113	0.118
TF38	0.100	0.104
TF39	0.097	0.103
TF46	0.162	0.157
TF47	0.162	0.160
TF48	0.165	0.160
TF49	0.158	0.155
TF56	0.249	0.261
TF57	0.235	0.240
TF58	0.233	0.235
TF59	0.232	0.254

Fig. 5. Bland-Altman plots of prediction results.

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Table 5. The Confidence Interval for the Mean Differences of Bland-Altman Plots

Model	Mean difference	Standard deviation	Standard error (SE)[a]	t value[b]	Confidence (SE*t)	Confidence intervals
190-400 nm	-0.003	0.007	0.002	2.09	0.003	-0.006 - 0.001

[a]Standard error = $\sqrt{SD^2/n}$. [b]t value with 19 degrees of freedom.

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