Original Article

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Development and validation of chemometrics-assisted spectrophotometric method for determination of clotrimazole in the presence of betamethasone valerate

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Abstract

A simple and accurate chemometrics-assisted spectrophotometric method was developed for determination of clotrimazole in the presence of betamethasone valerate. Partial-least square method (PLS-1) was selected for this objective. The PLS-1 models of clotrimazole and betamethasone valerate were separately developed on Unscrambler® program by using leave-one-out cross-validation of the calibration set samples. The resulted models were proved for their prediction abilities by using the test set samples, which were not contributed in models building step. The models were also validated for linearity, accuracy, and precision. The validation results showed that all of the parameters were within the acceptable limit. Finally, the developed and validated PLS-1 model was applied to quantitate clotrimazole in vaginal inserts. The determination results were appreciated and highly precised. Therefore, the PLS-1 model may be a simple and accurate alternative method to determine the amount of betamethasone and clotrimazole in formulations containing mixture of them.

Keyword: Clotrimazole, Betamethasone, PLS-1

1. INTRODUCTION

Clotrimazole, 1-[(2-chlorophenyl) diphenylmethy]-1H-imidazole, (Figure 1) is relative non-toxic synthetic imidazole derivative with broad-spectrum antimycotic activity. It has been well established that clotrimazole, available in the form of tablet, cream, and solution formulation, is used for the treatment of superficial mycoses in dermatology and gynaecology. Betamethasone valerate, [(8S,9R,10S,11S,13S,14S,16S,17R) -9-fluoro-11-hydroxy-17-(2-hydroxyacetyl) -10,13,16-trimethyl-3-oxo-6,7,8,11,12,14, 15,16-octahydrocyclopenta[a]phenanthren -17-yl] pentanoate, (Figure 1) is a potent glucocorticoids and commonly used to treat or reduce the symptoms of inflammation and allergy. Combination of clotrimazole and betamethasone has been widely used as topical antifungal and anti-inflammatory agents. Pharmaceutical formulations are usually complex mixtures of compounds comprising active pharmaceutical ingredient (API) and excipients. The effective assay method is important to prove the quality of pharmaceutical products for API as well as potential impurities and degradation products. Several methods have been described for quantitative determination of clotrimazole and betamethasone. High-performance liquid chromatography (HPLC) was used to determine clotrimazole in the presence of its degradation products or other drugs in the formulation.[1-3] Indirect titrimetric and extractive-spectrophotometric methods

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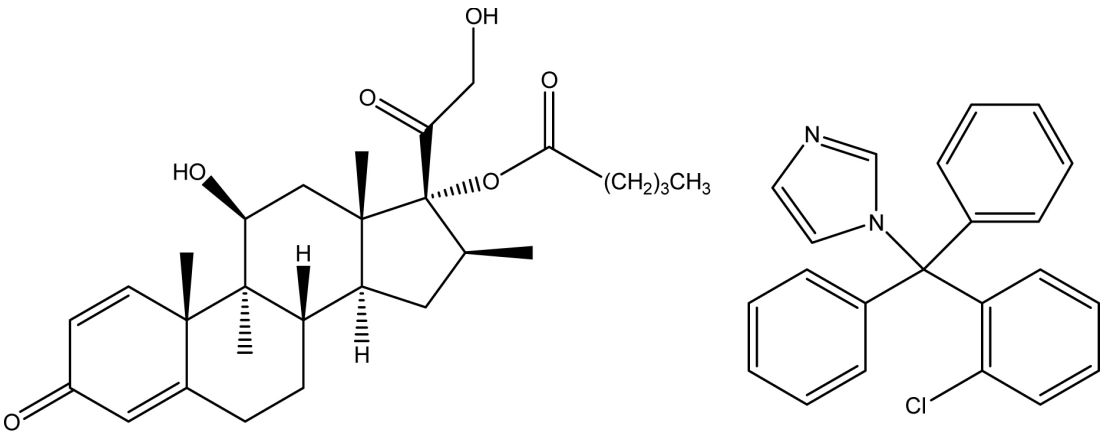
were used to determine clotrimazole and ketoconazole after reaction with triiodide ion and alizarin red S.[4] Differential pulse polarographic method was also used to determine clotrimazole after derivatization with Procion Red HE-3B.[5] Additionally, micellar electrokinetic chromatography was described for determination of clotrimazole, methylparaben and propylparaben in pharmaceutical preparation.[6] For combination of clotrimazole and betamethasone, micellar electrokinetic chromatography, HPLC, and liquid chromatography mass spectroscopic methods have been utilized.[3,7-8] In this study, chemometrics-assisted spectrophotometric method based on partial least square regression (PLS-1) was developed and validated for determination of clotrimazole in the presence of betamethasone valerate. The developed method was well applied to determine amount of clotimazole in the pharmaceutical products available in the market.



Betamethasone valerate **Clotrimazole**

Figure 1. Chemical structures of betamethasone valerate and clotrimazole

2. EXPERIMENTAL

2.1. Apparatus and software

The absorbance spectra were recorded by a Shimadzu (UV-160A) UV-Vis spectrophotometer (Bara scientific, Bangkok, Thailand) combined with a 1 cm quartz cell. Unscrambler® program was purchased from Charpa Techcenter Co., Ltd. (Bangkok, Thailand). Data analysis and PLS-1 modeling were performed by Unscrambler® program.

2.2. Reagents

Standards betamethasone valerate and clotrimazole were obtained from Sigma Chemical (S.M Chemical Co.Ltd., Bangkok, Thailand). Methanol and glacial acetic acid (Analytical grade) were purchased from RCI Labscan Limited (Bangkok,Thailand).

2.3. One component calibration

To find the linear concentration range of each drug, one component calibration was performed. Linearity ranges were studied in the concentration range of 13-67 μg/mL for betamethasone and 130-670 μg/mL for clotrimazole. Absorbance values were recorded at λ_{max} of each drug (240 nm for betamethasone and 260 nm for clotrimazole) in a 1-cm quartz cell. Methanol and glacial acetic acid (99:1, v/v) mixture was used as blank. Linearity range for each compound was determined by least-square linear regression of concentration and the corresponding absorbance.

2.4. Binary standards solutions

Two sets of standard solutions, calibration set and test set were prepared.

As shown in Tables 1 and 2, 16 solutions and 14 solutions were used in calibration set and test set, respectively. The concentrations of calibration set were selected by mean of central composite design (CCD) and those of test set were randomly selected.

3. RESULTS AND DISCUSSION

The resulting univariate calibration equations for the analytes at λ_{max} (240 nm for betamethasone and 260 nm for cotrimazole, respectively) were linear in the ranges of 13-67 µg/mL for betamethasone and 130-670 µg/mL for clotrimazole. To prevent obtaining solutions with overload absorbencies, the concentrations of betamethasone and clotrimazole in the mixtures were taken in the ranges of 0-43 and 0-240 µg/mL, respectively. The composition of the test samples (Table 2) was selected randomly according to the linear dynamic ranges.

Quantitative determination of combined drugs is usually performed by HPLC.[9-10] Spectrophotometric method is simple; however, sometimes it has a limitation for quantifying samples in the mixture. Since betamethasone and clotrimazole having UV-absorption properties in the same region (Figure 2), it was not allowed for simultaneous determination of these compounds by conventional univariate calibration methods. Therefore, multivariate calibration method i.e., PLS-1 was employed for simultaneous analysis of them.

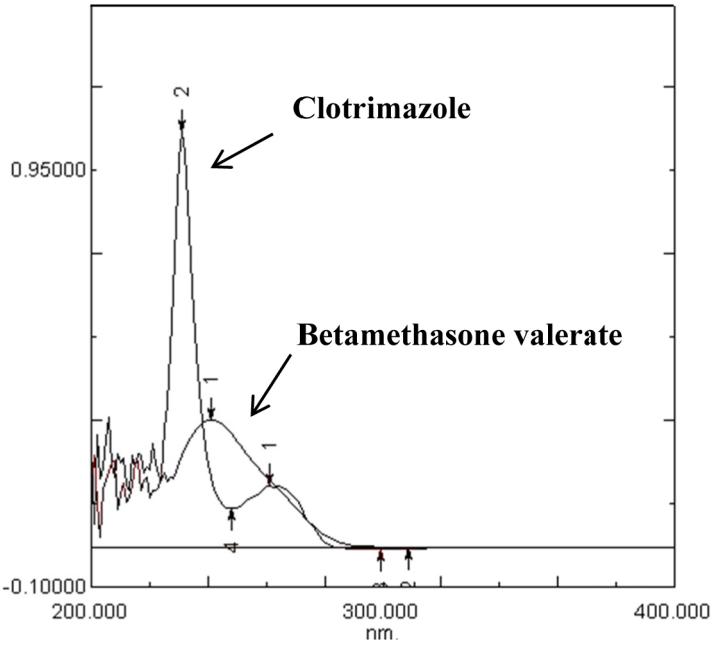


Figure 2. UV spectra of betamethasone valerate and clotrimazole

The standard solutions used in the multivariate calibration methods are mixtures of analytes. Accordingly, some important parameters should be considered in preparing of these standard solutions.[11] 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 calibration samples should not exceed the maximum absorbance reading of the instrument. Finally, the concentration of the prediction mixtures or test set (Table 2) should be the same range as that of the calibration mixtures.

Table 1. Compositions of CCD design for construction the determination models of betametasone and clotrimazole (calibration set)

Sample	Concentrations (μg/mL)	
	Betamethasone	Clotrimazole
1	24.0	152.0
2	24.0	152.0
3	24.0	152.0
4	24.0	152.0
5	24.0	0.0
6	24.0	0.0
7	0.0	152.0
8	0.0	152.0
9	43.0	152.0
10	36.0	215.0
11	24.0	240.0
12	10.0	215.0
13	6.0	152.0
14	10.0	90.0
15	24.0	65.0
16	36.0	90.0

Table 2. Compositions of samples for test set

Sample	Concentrations (μg/mL)	
	Betamethasone	Clotrimazole
1	13.0	107.0
2	10.0	100.0
3	9.0	140.0
4	20.0	200.0
5	15.0	220.0
6	10.0	160.0
7	30.0	175.0
8	25.0	230.0
9	37.0	190.0
10	30.0	120.0
11	25.0	95.0
12	28.0	70.0
13	23.0	150.0
14	25.0	150.0

The PLS-1 models for determination of betamethasone and clotrimazole were separately developed in Unscrambler® program. Model development was performed by using calibration standards. Leave-one-out cross-validation (LOO-CV) was used to validate PLS-1 models in model development and obtaining optimum latent variables (number of factors) of model. The resulting models were then validated to predict the concentration of analytes in a separate test set that did not contribute to the model development steps. The results of the prediction and the percentage of recoveries are represented in Table 3. As observed, there was a very good agreement between the predicted (calculated) and actual concentrations of drugs. The mean recoveries for betamethasone and clotrimazole were 100.4% and 100.0%, respectively. Then, the suitable models were validated to assure their intend purpose. Validation parameters such as linearity, accuracy, and precision (repeatability and intermediate precision) were evaluated. The results of models validation were presented in Table 4. All resulting validation parameters were within acceptable limit. Eventually, the developed and validated models were applied to determine the amount of clotrimazole in vaginal insert tablets. The assay results were presented in Table 5. As seen from this Table, the data indicate an excellent reproducibility of the prediction by the proposed models with the standard deviation (SD) less than 1.5.

Table 3. Test set determination results of the optimum PLS-1 models

Sample	Betamethasone			Clotrimazole		
	True conc. (μg/mL)	Predicted conc. (μg/mL)	% Recovery	True conc. (μg/mL)	Predicted conc. (μg/mL)	% Recovery
T1	13.0	12.8	98.6	107.0	106.3	99.4
T2	10.0	9.9	98.9	100.0	99.4	99.4
T3	9.0	8.8	97.6	140.0	139.5	99.7
T4	20.0	20.6	103.1	200.0	202.9	101.5
T5	15.0	14.9	99.8	220.0	211.6	96.2
T6	10.0	10.2	102.4	160.0	164.6	102.9
T7	30.0	30.2	100.6	175.0	178.3	101.9
T8	25.0	25.8	103.3	230.0	223.9	97.3
T9	37.0	37.3	100.9	190.0	194.0	102.1
T10	30.0	30.1	100.4	120.0	122.4	102.0
T11	25.0	25.1	100.5	95.0	97.2	102.3
T12	28.0	28.0	100.0	70.0	68.0	97.2
T13	23.0	23.0	100.1	150.0	149.1	99.4
T14	25.0	24.7	98.8	150.0	148.5	99.0
Average			100.4			100.0
% RSD			1.7			2.1

Table 4. Statistical parameters of the optimum PLS-1 model for betamethasone and clotrimazole determination

Parameter*	Betamethasone	Clotrimazole
Spectral range (nm)	245-320	260-320
Number of latent factors	2	3
r	0.9996	0.9989
RMSEP	0.4508	4.2870
Linearity	y = 1.0404 x – 0.6087 (r =0.9909)	y = 0.9385 x + 4.5547 (r = 0.9988)
Accuracy (% recovery)	96.0-96.6	96.8-101.3
Precision (repeatability, % RSD)	0.4-0.8	1.3-4.8
Precision (intermediate precision, % RSD)	0.6-1.9	1.5-3.4

* r = correlation coefficient; RMSEP = root mean square error of prediction

Table 5. Sample determination results

Sample	% Labeled amount
1	100.1
2	100.9
3	102.7
Average	101.2
SD	1.4

4. CONCLUSIONS

Partial least-square regression (PLS-1) models were successfully developed and validated for determination of betamethasone and clotrimazole combination. The resulting models were proved their efficiency by determination of betamethasone and clotrimazole contents in test set samples, which were not contributed in the calibration step. The models were also used to evaluate the content of clotrimazole in vaginal insert tablets. The assay results, expressed as percent labeled amount (%LA), were found acceptable. Even though the assay method for clotrimazole vaginal tablets in the current United State Pharmacopia (USP 37) is liquid chromatography[12], it is subjected to high technology instrument facility and time consuming. Fortunately, the results from this study showed that the chemometrics-assisted spectrophotometric method was simple, accurate, and may be used as an alternative method for determination of clotrimazole in the formulation mixtures.

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