SIMULTANEOUS DETERMINATION OF TETRACYCLINE AND CHLORTETRACYCLINE IN PHARMACEUTICAL DOSAGE FORM BY UV/VIS SPECTROPHOTOMETRY USING MULTIVARIATE REGRESSION

Đến tòa soạn 13-03-2021

Bui Phuong Thi, Pham Gia Bach, Bui Xuan Thanh, Ta Thi Thao
Faculty of Chemistry- VNU University of Science, Vietnam National University

TÓM TẮT

PHÂN TÍCH ĐỒNG THỜI TETRACYCLINE VÀ CHLORTETRACYCLINE TRONG DƯỢC PHẨM BẰNG PHƯƠNG PHÁP QUANG PHỔ HẤP THỤ PHÂN TỬ VÀ HỒI QUI ĐA BIẾN

Trong nghiên cứu này, phương pháp quang phổ hấp thụ phân tử (UV-Vis) kết hợp với thuật toán bình phương tối thiểu từng phần (PLS) đã được sử dụng để định lượng đồng thời tetracyclin khi có mặt chlortetracyclin ở dạng dược phẩm bào chế. Đây là phương pháp phân tích đơn giản, chính xác, nhạy và đáng tin cậy mà không cần qua bước tách loại hai chất có cấu trúc tương tự nhau trong quy trình phân tích. Khoảng tuyến tính để phát hiện tetracyclin và chlortetracyclin tương ứng là 0,5-10.0 µg/mL và 0,1-10.0 µg/mL.i. Để phân tích 2 chất trong mẫu thuốc, phổ UV của dung dịch chuẩn, mẫu chế tạo và mẫu thuốc được ghi trong khoảng từ 200 đến 326 nm với bước nhảy 2 nm trong môi trường HCl (pH 2). Hai ma trận hỗn hợp chuẩn chứa hai chất phân tích, trong đó 25 mẫu chuẩn làm ma trận chuẩn và 10 mẫu chuẩn dùng làm mẫu kiểm tra, được sử dụng để xây dựng và xác nhận giá trị sử dụng của mô hình đa biến. Mô hình sau khi tối ưu đã được áp dụng thành công để phân tích tetracyclin và chlortetracyclin trong mẫu dược phẩm đã chế tạo và mẫu thuốc chỉ chứa tetracyclin với độ chính xác đạt yêu cầu (giá trị thu hồi từ 88,2% đến 105,2%; (RSD <2%).

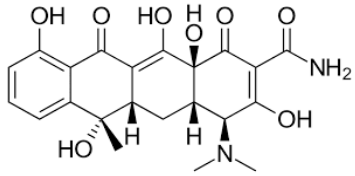
Từ khoá: *tetracyclin, chlortetrecyclin, quang phổ hấp thụ phân tử, bình phương tối thiểu từng phần.*

1. INTRODUCTION

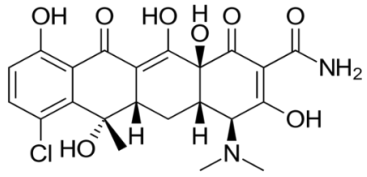
Two antibiotics tetracycline and chlortetracycline belong to the class of tetracyclines. The IUPAC names of tetracycline and chlortetracycline are *(4S,4aS,5aS,6S,12aR)-4-(dimethylamino)-1,6,10,11,12a-pentahydroxy-6-methyl-3,12-dioxo-4,4a,5,5a-tetrahydrotetracene-2-carboxamide and (4S,4aS,5aS,6S,12aR)-7-chloro-4-(dimethylamino)-1,6,10,11,12a-pentahydroxy-6-methyl-3,12-dioxo-4,4a,5,5a-tetrahydrotetracene-2-carboxamide*, respectively. The structure of tetracycline and chlortetracycline is shown in Figure 1. They are inexpensive antibiotics and have been widely used in the prophylactic and therapeutic treatment of human and animal infections as well as at the therapeutic level in the form of animal feed as stimulants to grow [1].

Liquid chromatography has been widely employed for tetracycline antibiotic determination [2]. One of the chromatographic techniques is the high-performance liquid chromatography which has been reported with satisfactory results [3]. The capillary zone electrophoresis methods have also been optimized for the simultaneous quantification of tetracyclines [4]. However, these methods

often require a number of chemical reaction or separation steps where sampling can increase the risk of human error in the results and analysis cost. Due to the similar structure of tetracycline and chlortetracycline, the molecular absorption spectrophotometry (UV-VIS) has never been applied to determine their concentrations in a mixture.



Tetracycline



Chlortetracycline

Figure 1. Structure of tetracycline and chlortetracycline

Chemometrics is considered a suitable alternative to analytical separation procedures. Therefore, different research groups have proposed the use of chemometric strategies for the simultaneous determination of antibiotics in pharmaceutical formulations by spectrophotometry. Multiple linear regressions are also applied to deal with overlapping spectral measurement signals of samples [5]. Partial Least Squares (PLS) is a multivariate calibration technique used in the resolution of multicomponent systems. The application of PLS algorithm is an excellent tool to eliminate the spectral interference in the quantification of the analytes.

In the present study, PLS has been used to identify and quantify tetracycline and chlortetracycline without separation steps. In mixtures of one or two components, the use of chemometric tools avoided spectral interference between the target substances. The predictive ability of calibration models was evaluated and showed satisfactory results [6,7].

2. EXPERIMENTAL

2.1. Apparatus and Software

Spectrophotometric measurements were performed on an UV-1601PC (Shimadzu) connected to a computer loaded with UV-Win PC software. All absorption spectra were saved and subsequently exported UV-Win software to the Microsoft Excel program for statistical manipulation. Matlab 2019a version software was employed to determine the concentration of combinations for calibration and validation sets.

2.2. Reagents and Samples

All chemicals were of analytical reagent grade. Tetracycline and chlortetracycline were obtained from the National Institute of Drug Quality Control (Hanoi, Vietnam). Double distilled water was used throughout the work. Commercial samples of antibiotics, tetracycline tablets labeled to contain 500 mg/tablet were purchased from the local pharmacies in Hanoi, Vietnam.

Standard solutions of tetracycline (1000 mg/L) and chlortetracycline (1000 mg/L) were prepared by dissolving the appropriate amounts of each analytical reagent in pure water. The solutions were stored and protected from light at 4°C. Working standard solutions were prepared daily by appropriate dilution in HCl medium (pH 2).

2.3. Procedure

a. One-component calibration.

This was examined in the concentration range of 0.5–10.0 µg/mL for tetracycline and 0.1–10 µg/mL for chlortetracycline. The wavelengths were 269.20 and 266.80 nm for tetracycline and chlortetracycline, recpectively. The linear dynamic range for each drug was studied by least-square linear (LSL) regression of concentration and the corresponding absorbance.

b. Construction of calibration and validation sets

The calibration and validation mixtures were prepared by combining the working standard solution of tetracycline and chlortetracycline in different ratios in their concentration linearity ranges. The concentration of mixtures were determined by general factorial design for calibration set (2 factors at 5 levels of tetracycline and chlortetracycline) and validation set (2 factors at 5 levels of tetracycline and 2 levels of chlortetracycline).

A total set of 25 calibration (Table 1) and 10 validation (Table 2) mixtures were independently prepared. The absorption spectra were recorded from 200 to 326 nm with an interval of 2 nm against distilled water as blank.

c. Analysis of the Marketed Formulations

Ten capsules were accurately weighed and finely powdered. A powder equivalent to tetracycline (500 mg) was accurately weighed and transferred into a 250 mL volumetric flask, and 100 mL of distilled water was added. The solution was well shaken and ultrasonicated for 15 min, and then was filtered in a 250 mL volumetric flask through a filter paper. The residue was washed three times with 10 mL distilled water. Finally, the filtered solution was diluted to 250 mL with distilled water. Suitable aliquots of the stock solutions were diluted with the solvent to obtain the appropriate working sample solution for UV measurements at the specified range.

Table 1. Composition of the calibration set for a standard solution.

Mix. no.	Tetracycline (µg/mL)	Chlortetracycline (µg/mL)
1	2	0.1
2	4	0.1
3	6	0.1
4	1	0.1
5	5	0.1
6	2	0.2
7	4	0.2
8	6	0.2
9	1	0.2
10	5	0.2
11	2	0.3
12	4	0.3
13	6	0.3
14	1	0.3
15	5	0.3
16	2	0.4
17	4	0.4
18	6	0.4
19	1	0.4
20	5	0.4
21	2	0.5
22	4	0.5
23	6	0.5
24	1	0.5
25	5	0.5

Synthetic mixture was used for the combination of working standard solutions while pharmaceutical mixture referred to the combination of sample solutions throughout this work.

3. RESULTS AND DISCUSSION

The representative absorption spectra of tetracycline and chlortetracycline are presented in Figure 2. Both spectra have a substantial overlapping region from 200 to 326 nm.

Due to the overlapping between two spectra of antibiotics, we have developed a multivariate model to spontaneously determine each antibiotic. Firstly, individual calibration curves were established to determine the linear range for each antibiotic. The calibration curves of difference in absorbance against concentration were plotted in Figure 3.

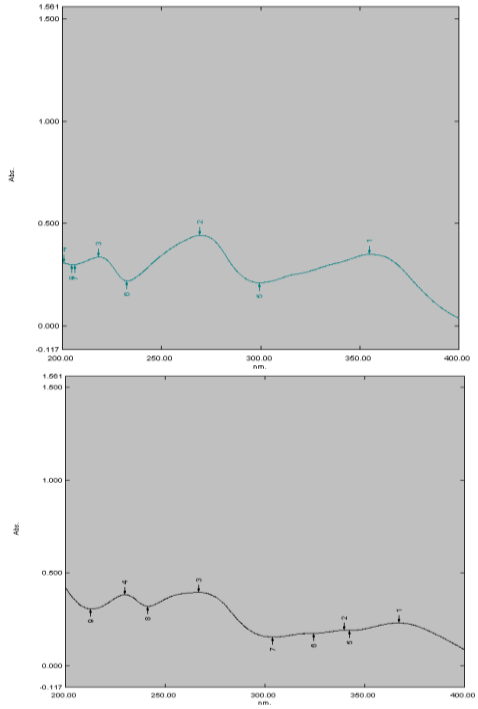


Figure 2. The representative spectra (absorbance vs. wavelength) of (A) tetracycline and (B) chlortetracycline from 200 to 326 nm

Secondly, the calibration and prediction sets were designed in 25 (Table 1) and in 10 (Table 2) artificial mixtures of tetracycline and chlortetracycline, respectively. The absorbance spectra which showed a significant overlap were recorded between 200 and 326 nm with an interval of 2 nm. Due to the overlapping between two absorbance spectra, the PLS method was used to establish multivariate

calibrations for both antibiotics. The model was built by Matlab 2019a software. To test the reliability of the multivariate model, 10 artificial mixtures (Table 2) was used to test. It shows that the concentrations of tetracycline and chlortetracycline calculated by the model are similar to the actual concentrations with the percent recovery of 99% to 116%.

The predictive ability of models was evaluated by the root-mean-square error (RMSE) of test , the RMSE of standard, and correlation coefficient (R^2) (Table 2). The RMSE of standard generated from the validation set is the estimated prediction error that accurately reflects all sources of variability in the calibration method. To validate the model, both RMSE of test and standard must be as low as possible for a model [8]. For tetracycline detection, RMSE of test (RMSEt) was calculated as 0.01 while RMSE of standard (RMSEs) as 0.021. A correlation coefficient of 0.999 was obtained for tetracycline in the validation set samples by PLS-optimized models, demonstrating a good predictive ability of the models.

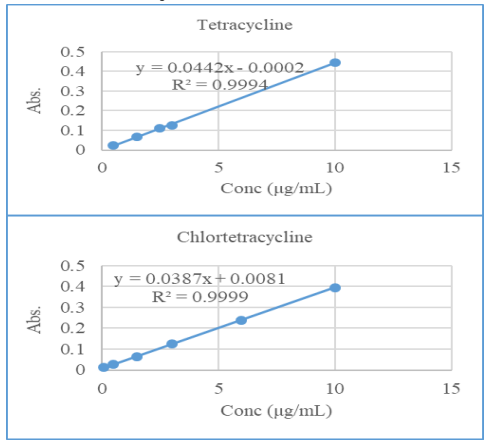


Figure 3. The plots of Beer – Lambert's law of absorbance for (A) tetracycline at 269.20 nm and (B) chlortetracycline at 266.80 nm

Table 2. Composition of validation set and predicted results obtained in synthetic mixtures by PLS methods.

Concentration (µg/mL)		PLS			
Tetracycline	Chlortetra-cycline	Tetracycline		Chlortetracycline	
		Found (µg/mL)	% recovery	Found (µg/mL)	% recovery
2.00	0.10	1.99	99.6	0.09	88.0
4.00	0.10	3.99	99.8	0.12	115.7
6.00	0.10	5.99	99.9	0.10	99.3
1.00	0.10	1.01	101.1	0.12	116.2
5.00	0.10	5.00	99.9	0.08	79.8
2.00	0.20	2.02	100.9	0.20	100.9
4.00	0.20	4.01	100.25	0.20	100.9
6.00	0.20	5.99	99.85	0.20	101.1
1.00	0.20	1.00	100.3	0.20	99.5
5.00	0.20	5.01	100.2	0.21	105.2
R^2		0.999		0.959	
RMSEt		0.010		0.011	
RMSEs		0.021		0.016	

The proposed methods were applied for the assay of tetracycline in the presence of chlortetracycline in pharmaceutical dosage form. The assay results are shown in (Table 3) indicating a good agreement with the concentration taken for the formulations.

Table 3. Assay results of tetracycline in the presence of chlortetracycline in pharmaceutical dosage form by developed PLS method.

Actual Concentration (mg/capsule)	Tetracycline	
	Predicted (mg/tablet)	Recovery (%)
500	485 ± 15	97.04

4. CONCLUSION

A simple and reliable spectrophotometric method has been developed in this work for the spontaneous detection of two antibodies. The use of chemometric techniques such as PLS could reduce sample pretreatment steps which are traditionally required for the proper determination of multiple components in real samples.

The results were obtained from the analyses of commercial sample to confirm the predictive ability of the method to eliminate spectral interferences without the use of separation steps. The flexibility of the method could be further applied to analyze samples containing a variable number of tetracycline type antibiotics such as doxycycline, minocycline, and tigecycline.

REFERENCES

[1] Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP, et al., *Global trends in antimicrobial use in food animals*. Proc Natl Acad Sci USA, 112(18), 2015, 5649–54.

[2] Amir GKazemifard, Douglas EMoore; *Evaluation of amperometric detection for the liquid-chromatographic determination of tetracycline antibiotics and their common contaminants in pharmaceutical formulations*. Journal of Pharmaceutical and Biomedical Analysis, 16(4) 1997, 689-696.

[3] FridaDarghouth; LotfiMonser; *Rapid liquid chromatographic method for simultaneous determination of tetracyclines antibiotics and 6-Epi-doxycycline in pharmaceutical products using porous graphitic carbon column*; Journal of Pharmaceutical and Biomedical Analysis, 23(2-3), 2000, 353-362.

[4] Mónica Cecilia VargasMamani et al., *Simultaneous determination of tetracyclines in pharmaceuticals by CZE using experimental design*. Talanta, 70(2), 2006, 236-243.

[5] Tadele Eticha et al., *Chemometric-Assisted Spectrophotometric Method for the Simultaneous Determination of Ciprofloxacin and Doxycycline Hyclate in Pharmaceutical Formulations*. Journal of Analytical Methods in Chemistry, 11, 2018, 1-5.

[6] Pedro LuisLópez-de-Alba et al., *Spectrophotometric determination of Allura Red (R40) in soft drink powders using the universal calibration matrix for partial least squares multivariate method*. Analytica Chimica Acta, 30(1), 1996; 19-29.

[7] Amador-Hernández J. et al., *Resolution of a multicomponent polycyclic aromatic hydrocarbon system in micellar media by linear variable angle fluorescence applying distinct chemometric techniques*, Analyst, 123(11), 1998, 2235-2241.

[8] P. Gemperline (2006) *Practical Guide to Chemometrics*; Taylor & Francis.