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FTIR Combined with Chemometrics for Fast Simultaneous Determination of Penicillin and Cephalexin in Pharmaceutical Tablets

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Abstract

In this research, the potential of combining chemometrics with FTIR techniques to provide a rapid and simultaneous quantitative analyses method for determination penicillin, cephalexin is studied. Unlike other methods, FTIR is considered as a time saving method due to its non-destructive and simple sample preparation. Due to the similarity of infrared spectral, PLS and PCR couples with spectral treatment techniques are applied to make the calibration model for penicillin and cephalexin determination at the same time. The result is also validated in term of RMSEP and R^2 value using validation set. The FTIR combined with PLS method shows the best results.

Key words: Chemometrics; FTIR method; penicillin; cephalexin.

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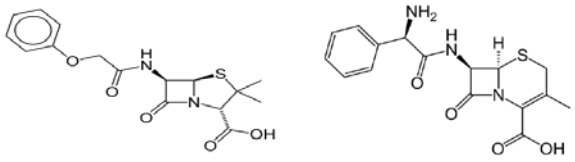
1. Introduction

According to WHO, 10% of world drug market are dominated by counterfeit, causing nearly 200,000 deaths per year [1]. In Vietnam, National Institute of drug quality control reported that in 2011, 31,000 drug samples were collected for testing purpose, the result showed that more than 1,000 samples were unqualified [2]. Therefore, development of a quick and accurate analysis method for determination of counterfeit is urgent problem in Vietnam. Antibiotics are substances which have ability to kill or to inhibit the growth of bacteria. Therefore, since the beginning of 20th century, they were widely used in treatment or prevention for patient who are suffered bacterial infection. β-lactam is a group of antibiotic that contains β-lactam ring in structure. Penicillin (Figure 1a) and cephalexin (Figure 1b) are two the most widely used among 4 types of β-lactam antibiotic [3].



(a) (b)

Figure 1: Chemical structures of (a) Penicilin and (b) Cephalexin

Due to their popularity, antibiotics in Penicillin and Cephalosporin groups are also easy to be counterfeited and causing serious health problem for patient. Because of their similarity of molecular structures and complicated matrix background, it is a challenge for us to do rapidly simultaneous determination of them. In Vietnamese Pharmacopeia [4], the high performance liquid chromatography (HPLC) technique has been designed as a standard method for the analysis of antibiotics in formulated and unformulated samples. Beside that, capillary zone electrophoresis [5], spectrophotometric [6]. However, determination of antibiotics by using these methods requires prior pre-sampling and separation before analysis. FT-IR spectroscopy is a fast and non-destructive technique, sensitive, and simple in sample preparation, based on the vibrational spectroscopy [7]. In this study, an FTIR method in combination with chemometrics was developed for quick and simultaneous determination of two selected β-lactam antibiotics including penicilin (amoxiclin group) and cephalexin (cephalosporin group) in pharmaceutical drugs. The study confirmed a very useful model that can be applied for simultaneous determination of β-lactam antibiotics by one model.

2. Materials and methods

2.1. Sample preparation

For calibration model, 24 samples containing penicillin and cephalexin, and other excipients at different concentration ranges as powder form in mixture with KBr were prepared. For validation set, 10 independent samples which were different from calibration set with known concentrations were also made. All samples were subjected to FTIR analysis using Agilent Cary FTIR 600. FT-IR spectra were scanned between 3600 cm^{-1} and 2800 cm^{-1}, by averaging 32 scans for each spectrum with a resolution of 4 cm^{-1}.

2.2. Statistical analysis

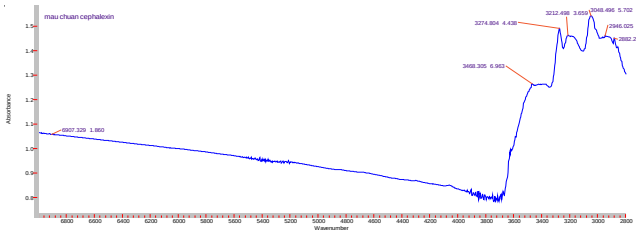
Multivariate calibration of partial least square (PLS) and principle component regression (PCR) ware performed using Matlab 2015, employing the raw data and first and second derivative-transformed spectra. The values of coefficient of determination (R^2) and root mean square error of calibration (RMSEC) were used as performance criteria for calibration model [8]. RMSEC = $\sqrt{\frac{\sum_{i=1}^{n}(actual-calculated)^2}{N-f-1}}$

Smaller RMSEC value, less uncertainty of calibration. Also, R^2 values and root mean square error of prediction (RMSEP) together can show how well the developed model for quantitative analysis of new samples; the lower the RMSEP value, the better the prediction performance of the model. RMSEP = $\sqrt{\frac{\sum_{i=1}^{m}(actual-calculated)^2}{M-1}}$

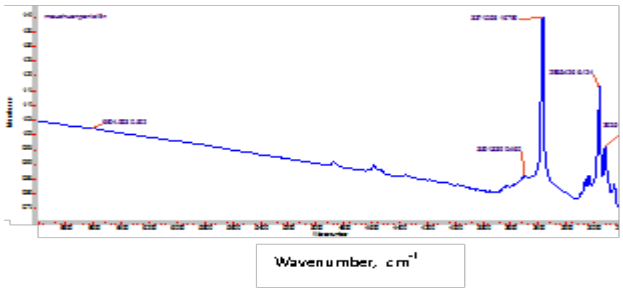
The term “actual” means the known concentration of selected sample; and the term “calculated” refers to a concentration calculated by the model using spectral data; N and M are the number of samples used in the calibration and validation sets, respectively; f is the number of factors used in the calibration model by PCA or PLS [9].

3. Results and discussion

3.1. Spectral regional selection



a



b

Figure 2: FTIR spectra of cephalexin (a) and penicillin (b) in near infrared region (7500 cm^{-1} – 2800 cm^{-1}).

Figure 2 shows FTIR spectra of penicillin (PEN) and cephalexin (CEF) in near infrared region (7500 cm^{-1} - 2800 cm^{-1}). Region from 4000 cm^{-1} to 1000 cm^{-1} is known as the functional group region and the region below 1000 cm^{-1} is called the fingerprint region [8]. Peaks in range of 3500 cm^{-1} to 3200 cm^{-1} are caused by single bonds N-H, and O-H (in carbocyclic acid) stretching vibration; the stretching of C-H in aromatics cause peaks from 3100 cm^{-1} to 3000 cm^{-1}, and from 3000 cm^{-1} to 2850 cm^{-1} are caused by single bonds C-H [9]. Spectral region (frequency) selection is one of the most important problems in FTIR analysis because the chosen frequency regions must be describe the most characteristics of analytes and not include interfering data for the analyses [10]. After selection, the spectral regions from 3500 cm^{-1} to 2800 cm^{-1} were chosen for further making PLS and PCR calibration models. Also, result shows the overlap of the spectra due to similar molecular structure, causing problem for simultaneous determination of two compounds. Therefore, chemometrics must be applied to solve this problem [11].

3.2. Optimization of Spectral Data

The optimization process was continued by investigating several treatments of FTIR spectra (normal and derivatives) using selected frequency. The first derivative spectra treatment eliminates the common intensity effect of FTIR spectra and can simplify the baseline selection. And, the second derivative can remove the slope effect [12]. However, derivation treatments can affect the measurement sensitivity.

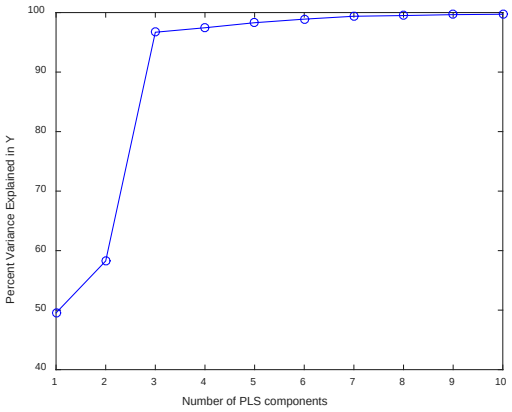


Figure 3: Percent variance explained by each component.

Figure 3 indicates that three first components already explained for more than 95% variance, and further PLS, PCR models can conducted with three first components. The selection of model was based on the highest coefficient of determination (R^2) and the lowest root mean standard error of calibration (RMSEC) values (Table 1). The highest R^2 value (0.986) and lowest RMSEC value (0.750) of PLS model for PEN determination were obtained, and the highest R^2 and lowest RMSEC value found for CEF determination respectively were 0.963 and 1.160. The highest R^2, and lowest RMSEC values obtained from PCR calibration model for PEN and CEF determination respectively were 0.967, 1.140 and 0.943, 1.428. Relying the highest value of R^2 and the lowest value of RMSEC as shown in table 1, PEN and CEF were determined by using PLS calibration model combined with first derivative spectra, meanwhile normal spectra were applied for PCR model.

Table 1: The statistical parameters using chemometrics for simultaneous determination of PEN and CEF

Analytes	Model	PEN		CEF	
Spectral treatments		**R^2**	**RMSEC**	**R^2**	**RMSEC**
Normal	PLS	0.973	1.050	0.950	1.340
1st Deri.		0.986	0.750	0.963	1.160
2nd Deri.		0.948	1.440	0.912	1.770
Normal	PCR	0.967	1.140	0.943	1.428
1st Deri.		0.896	2.041	0.896	1.936
2nd Deri.		0.397	4.909	0.443	4.471

3.3. Validation of the model

The calibration models were further validated using 10 independent prepared samples with known concentrations. The performance of PLS, PCR model with all spectra treatment techniques were shown in table 2 in term of R^2 and RMSEP value. With the validation set, the performance of PLS and PCR models showed good results in combination with non-derivative spectra treatment. For both PEN and CEF determination, the PCR model with non-derivative treatment showed a slightly better results in term R^2 value. However in term of RMSEP value, the results obtained from PLS model were better in both cases. Therefore, for quick and simultaneously quantification both PEN and CEF in drug the non-derivative spectra treatment and the 3500 cm^{-1} - 2800 cm^{-1} spectra region were selected for this research.

Table 2: The performance of validation model used for simultaneous prediction of PEN and CEF

	Spectral treatments	PEN		CEF	
		R^2	RMSEP	R^2	RMSEP
PLS	Normal	0.946	1.890	0.934	1.470
	1st Deri.	0.936	2.100	0.911	1.760
	2nd Deri.	0.742	3.690	0.730	3.080
PCR	Normal	0.949	2.060	0.935	1.570
	1st Deri.	0.929	2.760	0.949	2.140
	2nd Deri.	0.005	5.900	0.060	5.000

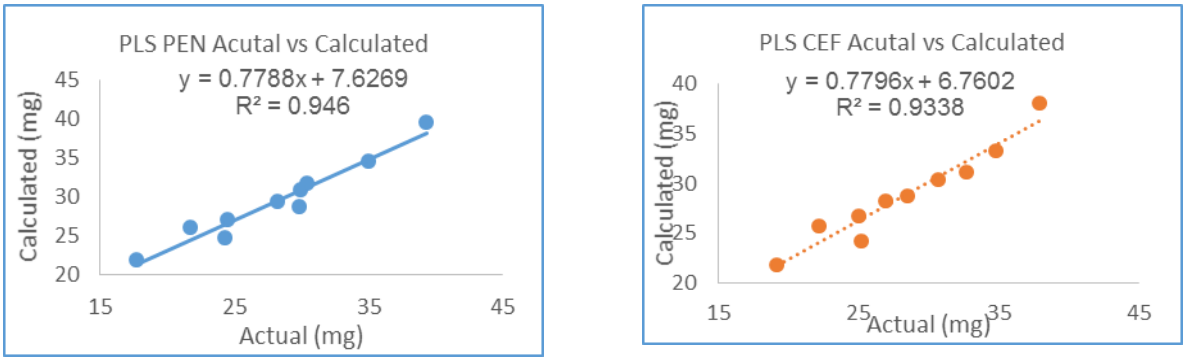


Figure 4: PLS scatter plot for the relationship between actual values and FTIR calculated value of penicillin and cephalexin.

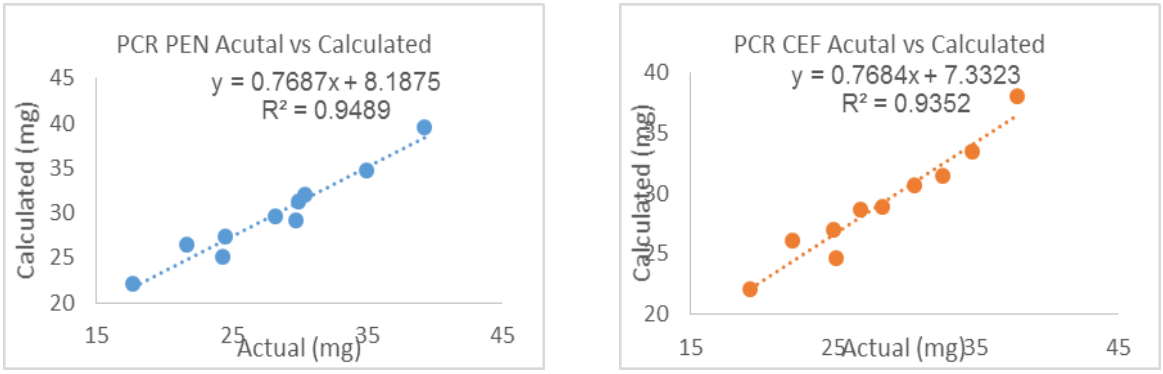


Figure 5: PCR scatter plot for the relationship between actual values and FTIR calculated value of Penicillin and Cephalexin

The PLS scatter plots the relationship between actual value (x-axis) and FTIR predicted value (y-axis) of PEN, CEF measured using non-derivative FTIR spectra is shown in figure 4, and figure 5 shows the relationship using PCR model.

The low value of RMSEP and the high value of R^2 indicate that FTIR spectroscopy combined with chemometrics of PLS regression and appropriate spectral treatments is a very good technique for simultaneously quantification of SFG and SFM in drug.

4. Conclusion

It can be concluded that FTIR spectroscopy combined with partial least square regression with non-derivative spectra at frequency region of 3500 cm^{-1} - 2800 cm^{-1} can be used for simultaneously qualification and quantification both penicillin, and cephalexin in drug.

The R^2 values obtained for the relationship between actual and FTIR predicted values of PEN and CEF were 0.973, and 0.950 with RMSEC values of 1.050, and 1.340, respectively. The developed method is fast, with no excessive sample preparation, and is not involving the use of reagents or chemicals.

5. Competing Interests

The authors declare that they have no competing interests

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References

[1] M. Alcalà, M. Blanco, D. Moyano, N. Broad, N. O'Brien, D. Friedrich, F. Pfeifer, H. Siesler, "Qualitative and quantitative pharmaceutical analysis with a novel handheld miniature near-infrared spectrometer", J. Near Infrared Spectrosc. 21 (6), pp. 445, 2013,

[2] World Health Organization, "Counterfeit and Substandard Drugs in Myanmar and Viet Nam". Essential Drugs and Other Medicines , Geneva, 1999

[3] J. D. Campagna, M. C. Bond, E. Schabelman, B.D. Hayes, "The use of cephalosporins in penicillin-allergic patients: a literature review". J Emerg Med, vol. 42(5), pp. 612-20, 2012.

[4] Vietnamese Pharmacopeia. Edition 2010

[5] B. Simon, G. Hancu, A. Gyéresi, "Application of capillary electrophoresis to the simultaneous determination and stability study of four extensively used penicillin derivatives". Brazilian Journal of Pharmaceutical Sciences, vol. 50, n. 3, pp. 521-527, 2014.

[6] S. M. Ali , A. A. Elbashir, H. Y. Aboul-Enein, "Spectrocopic methods for analysis of cephalosporins in pharmaceutical formulations," World Journal of Analytical Chemistry. Vol. 3, No. 1A, pp. 21-32, 2015.

[7] Y. Roggo, P. Chalus, L. Maurer, C. Lema-Martinez, A. Edmond, N. Jent, "A review of near infrared spectroscopy and chemometrics in pharmaceutical technologies", Journal of Pharmaceutical and Biomedical Analysis, 44, pp. 683-700, 2007.

[8] D. Pavia, "Introduction to spectroscopy : a guide for students of organic chemistry", Brooks Cole: South Melburne, 2001.

[9] D. Ami, P. Mereghetti, S. M. Doglia, "Multivariate Analysis for Fourier Transform Infrared Spectra of Complex Biological Systems and Processes". Multivar. Anal. Manag. Eng. Sci. pp.189-220, 2012.

[10] A. Rohman, D. L. Setyaningrum, S. Riyanto, "FTIR spectroscopy combined with partial least square for analysis of red fruit oil in ternary mixture system", Int. J. Spectrosc., pp.1-5, 2014.

[11] Y. Roggo, P. Chalus, L. Maurer, C. Lema-Martinez, A. Edmond, N. Jent, "A review of near infrared spectroscopy and chemometrics in pharmaceutical technologies", J. Pharm. Biomed. Anal., 44, pp. 683-700, 2007.

[12] M. J. Lerma-García, G. Ramis-Ramos, J. M. Herrero-Martínez, E. F. Simó-Alfonso, "Authentication of extra virgin olive oils by Fourier-transform infrared spectroscopy", Food Chem. 118 (1), pp. 78-83, 2010.

[13] A. Rohman, Y. B. Che Man, A. Ismail, H. Puziah, FTIR spectroscopy combined with multivariate calibration for analysis of cod liver oil in binary mixture with corn oil. Int. Food Res. J. 18 (2), 757-761, 2011.