SPECTROMETRIC DETERMINATION OF LEVOFLOXACIN AND NORFLOXACIN IN TABLETS BY MULTIVARIATE CALIBRATION APPROACH

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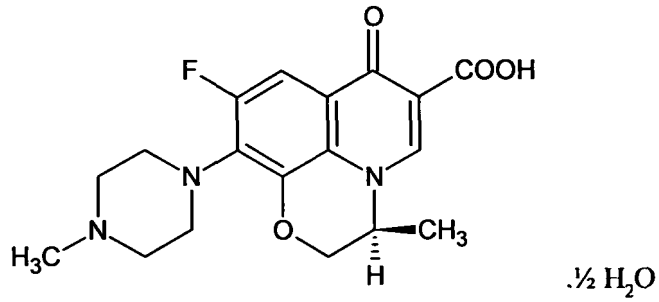
ABSTRACT

Three multivariate calibration-prediction techniques, classical least squares (CLS), inverse least squares (ILS) and partial least squares (PLS) were applied to the spectrometric multicomponent analysis of the drug containing levofloxacin (LEVO) and norfloxacin (NORF) without any separation step. The selection of variables was studied. A series of synthetic solution containing different concentrations of LEVO and NORF were used to check the prediction ability of the CLS, ILS and PLS. The results obtained in this investigation strongly encourage us to apply these techniques for a routine analysis and quality control of the two drugs.

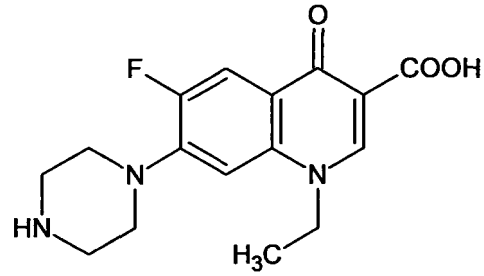
Keywords: levofloxacin, norfloxacin, spectrometry, multivariate calibration

INTRODUCTION

Fluoroquinolones are a widely used class of antibiotics, whose utilization in industrial farming has been one of the main contributing factors towards the emergence of resistant bacteria. Levofloxacin (LEVO) and norfloxacin (NORF) are floroquinolone carboxylic acid, currently used as a broad spectrum antibacterial drug /1/ against gram-negative and gram-positive aerobic pathogens, and considered to be the first commercially available member of the modern fluoroquinolones. On the other hand, these agents are becoming important for quality control in the commercial pharmaceutical tablets. Their chemical structures are presented in Fig.1.



Levofloxacin



Norfloxacin

Fig. 1: Chemical structures and names of the studied compounds

During the last decade the powerful chemometric methods classical least-square (CLS), inverse least-square (ILS), and partial least-squares (PLS) were used in spectral data analysis for the mixtures containing two or more compounds with overlapping spectra /2-4/. These methods have a wide range of applications, e.g. spectrometric /5,6/, chromatographic /7/ and electrochemical /8/ quantitative analysis.

Several methods have been reviewed in the literature for the analysis of LEVO and NORF. A number of spectrometric methods for the determination of fluoroquinolones /9,12/ were reported. The polarographic /13/, stripping voltammetric /14/ and chromatographic /15,16/ methods, which involve multiple steps and sometimes utilize expensive and sophisticated instruments, were also studied.

The multivariate calibration techniques use full spectrum, full automation, multivariate data analysis and the reduction of noise and the advantages of the selection calibration model. In addition these multivariate calibrations do not need any separation procedure, they are very cheap, very easy to apply and very sensitive. For these reasons these multivariate techniques are

popular today.

In this study three chemometric methods were applied to analyse the synthetic mixtures and tablets consisting of LEVO and NORF in the presence of interferences of the absorption spectra. The application of chemometrics allows the interpretation of multivariate data and is vital to the success of the simultaneous determination of the clinical drugs.

EXPERIMENTAL

Apparatus

A Shimadzu (Model UV-1700) UV-Visible spectrometer (Shimadzu, Kyoto, Japan), equipped with 1cm matched quartz cells, was used for spectrometric measurements.

Standard solutions

All materials used were of analytical grade. Stock solutions of 100 mg/100 mL LEVO and NORF were prepared in 0.05 M HCl. A training set containing 0-14 μg/mL LEVO and 0-26 μg/mL NORF in possible proportions and twelve synthetic mixture solutions as a validation set in the concentration range of 4-14 μg/mL LEVO and 6-26 μg/mL NORF were prepared by using the above stock solutions. The solutions were stable for at least two weeks if they had been stored in a cool (< 25°C) and dark place.

Pharmaceutical preparations

Two commercial preparations; Noroxin® tablet (produced by Merck Sharp & Dohme Pharm. Ind., Turkey, containing 400 mg norfloxacin) and Tavanic® tablet (produced by Aventis Pharm. Ind., containing 500 mg levofloxacin) per tablet were analyzed by the proposed chemometric techniques.

Procedure for dosage forms

Twenty tablets were accurately weighed and powdered in a mortar. A sample containing LEVO and NORF equivalent to half of the contents of the

tablets was dissolved 0.05M HCl and made up in 100 mL calibrated flasks. The content of the flask was sonicated for about 15 min and filtrated into a 100 mL volumetric flask through 0.20 μm membrane filter. The resulting solution was diluted 1:100 with the same solvent. All the techniques were applied to the final solution.

CHEMOMETRICS METHODS

CLS

Classical least-squares, sometimes known as K-matrix calibration, are so called because, originally, it involved the application of multiple linear regressions (MLR) to the classical expression of the Beer-Lambert Law of spectroscopy:

$$A = K \times C \quad (1)$$

The above matrix equation can be written as a linear equation system:

$$A_1 = K_{11}C_1 + K_{12}C_2 + \ldots\ldots + K_{1c}C_c$$
$$A_2 = K_{21}C_1 + K_{22}C_2 + \ldots\ldots + K_{2c}C_c$$
$$\ldots\ldots \quad \ldots\ldots \quad \ldots\ldots \quad \ldots\ldots\ldots$$
$$A_n = K_{n1}C_1 + K_{n2}C_2 + \ldots\ldots + K_{nc}C_c$$

where A_n represents the absorbance at the n^{th} wavelength, K_{nc} is the calibration coefficient corresponding to the c^{th} component at the n^{th} wavelength, while, C_c is the concentration of the c^{th} component.

ILS

Inverse least-squares, sometimes known as P-matrix calibration, are so called because, originally, it involved the application of multiple linear regressions (MLR) to the inverse expression of the Beer-Lambert Law of spectroscopy:

$$C = P \times A \quad (2)$$

Equation (2) is a matrix equation. For clarity, we can expand this equation to give:

$$C_1 = P_{11}A_1 + P_{12}A_2 + ... + P_{1w}A_w$$
$$C_2 = P_{21}A_1 + P_{22}A_2 + ... + P_{2w}A_w$$
$$... \quad ... \quad .... \quad .....$$
$$C_c = P_{c1}A_1 + P_{w2}A_2 + ... + P_{cw}A_w$$

where A_w is the absorbance at the w^{th} wavelength, P_{cw} is the calibration coefficient for the c^{th} component at the w^{th} wavelength and C_c is the concentration of the c^{th} component.

PLS

In the UV-Vis spectra, the absorbance data (A) and concentration data (C) are mean centered to give data matrix A_o and vector C_o. The orthogonalized PLS algorithm has the following steps. The loading weight vector W has the following expression:

$$W = \frac{A_o^T C_o}{C_o^T C_o}$$

The scores and loadings are given by:

$$t_1 = A_o W \; ; \; P_1 = \frac{A_o^T t_1}{t_1^T t_1} \; ; \; q_1 = \frac{C_o^T t_1}{t_1^T t_1}$$

The matrix and vector of the residuals in A_o and C_o are:

$$A_1 = A_o - t_1 p_1^T$$
$$C_1 = C_o - t_1 q_1^T$$

From the general linear equation, the regression coefficients were calculated by:

$$b = W (P^T W)^{-1} q$$
$$a = C_{mean} - A_{mean}^T b$$

The constructed calibration equation is used for the estimation of the compounds in the samples.

RESULTS AND DISCUSSION

Fig.2. shows the absorption spectra for LEVO and NORF and their mixture in 0.05 M HCl. In order to build the three chemometric calibrations, a training set was randomly prepared by using the standard mixture solution containing 0-14 μg/mL LEVO and 0-26 μg/mL NORF in the variable proportions as shown in Table 1. The absorbance data matrix were obtained by measuring at the 13 wavelengths with the intervals $\Delta\lambda = 5$ nm in the 260 – 315 nm spectral region. The prepared calibrations of three techniques using the absorbance data sets were used to predict concentration of the unknown values of LEVO and NORF in their mixture. Linearity range was 4-14 μg/mL for LEVO and 6-26 μg/mL for NORF in the multivariate calibration proposed.

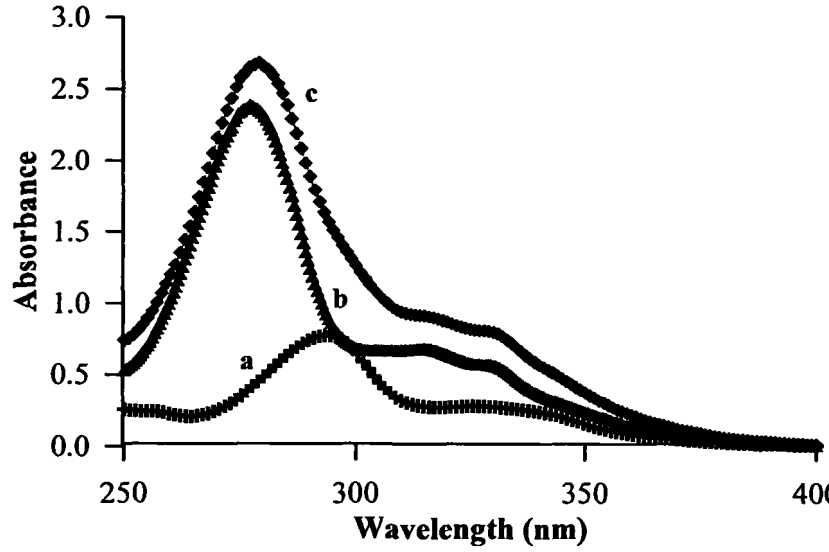


Fig. 2: Original absorption spectra of: (a) 8 μg/mL LEVO; (b) 18 μg/mL NORF; and (c) their mixture in 0.05M HCl

Table1
Composition of a training set of standard synthetic mixtures containing two drugs

Standard No	LEVO (μg/mL)	NORF (μg/mL)
1	4	26
2	6	22
3	8	18
4	10	14
5	12	10
6	14	6
7	14	0
8	12	10
9	10	14
10	8	18
11	6	22
12	0	26

A calibration for each technique was computed in the MAPLE 7.0 and PLS Toolbox 4.0 software by using a set consisting of two drugs and their absorbance data. The multivariate calibrations of three techniques were used to predict the unknown concentrations of LEVO and NORF in the samples.

Some statistical parameters were given for the validation of the constructed calibrations for the training set and synthetic binary mixtures of both drugs.

The application competence of a calibration model can be explained in several ways. We can also examine these results numerically. One of the best ways to do this is by examining the predicted residual error sum-of-squares or PRESS. To calculate PRESS we compute the errors between the expected and predicted values for all the samples, square them, and sum them together.

$$\text{PRESS} = \sum_{i=1}^{n} (C_i^{added} - C_i^{found})^2$$

Strictly speaking, this is not a correct way to normalize the PRESS values when not all of the data sets contain the same number of samples. If we want to correctly compare PRESS values for data sets that contain differing numbers of samples, we should convert to standard error of prediction (SEP), which is given by following formula.

$$SEP = \sqrt{\frac{\sum_{i=1}^{n}(C_i^{added} - C_i^{found})^2}{n-1}}$$

where C_i^{added} the added concentration of drug is, C_i^{found} is the found concentration of drug and n is the total number of the synthetic mixtures. The SEP can provide a good measure of how well, on average, the calibration model performs. Often, however, the performance of the calibration model varies depending on the analyte level.

In the application of three chemometric techniques to the synthetic mixtures containing two drugs in variable compositions, the mean recoveries and relative standard deviations for CLS, ILS and PLS were found to be 101.9 and 1.43%, 100.7 and 1.58%, 100.9 and 0.88%, respectively for LEVO and 99.3 and 1.44%, 99.8 and 1.68%, 100.6 and 0.77%, respectively for NORF (Table 2).

Table2

Results obtained for LEVO and NORF indifferent synthetic mixtures by using proposed techniques

Mixtures added (μg/mL)		Recovery (%)					
		CLS		ILS		PLS	
LEVO	NORF	LEVO	NORF	LEVO	NORF	LEVO	NORF
4	18	99.5	97.8	102.8	98.0	101.5	99.6
6	18	103.8	99.3	101.1	98.7	102.3	100.1
8	18	101.6	97.1	99.5	98.1	100.2	101.5
10	18	101.0	98.2	101.2	100.2	100.8	100.2
12	18	103.1	99.2	102.5	98.5	101.3	102.0
14	18	104.1	98.3	102.0	100.7	102.5	100.2
6	6	100.7	101.5	98.1	102.1	99.8	101.2
6	10	100.5	100.1	101.3	100.0	100.2	100.2
6	14	101.5	98.0	100.3	97.8	100.2	99.8
6	18	102.3	99.7	99.3	99.8	101.7	101.2
6	22	103.3	101.1	101.8	102.8	100.4	101.3
6	26	101.3	101.0	98.3	101.5	101.0	100.2
Mean	101.9	99.3	100.7	99.8	100.9	100.6	
RSD*		1.43	1.44	1.58	1.68	0.88	0.77

*RSD: Relative Standard Deviation.

According to the added concentration and the concentration found in samples, the SEP and PRESS values of CLS, ILS and PLS techniques were calculated as 0.264 and 0.626, 0.165 and 0.245, 0.146 and 0.191 respectively for LEVO, 0.313 and 0.880, 0.339 and 1.035, 0.216 and 0.419 respectively for NORF (Table 3).

Table 3
Statistical parameters in the calibration-prediction

Parameter	Method	LEVO	NORF
PRESS	CLS	0.626	0.880
	ILS	0.245	1.035
	PLS	0.191	0.419
SEP	CLS	0.264	0.313
	ILS	0.165	0.339
	PLS	0.146	0.216
r*	CLS	0.999	0.998
	ILS	0.999	0.997
	PLS	0.999	0.999
Intercept	CLS	-0.186	-0.214
	ILS	-0.169	-0.315
	PLS	-0.108	-1.10^{-5}
Slope	CLS	1.047	1.005
	ILS	1.031	1.017
	PLS	1.025	1.007
RSD	CLS	0.095	0.079
	ILS	0.030	0.107
	PLS	0.035	0.044

*Regression coefficient

The linear regression analysis of the added concentration and the concentration found in the synthetic mixtures were realized for each drug and for each calibration technique. In this regression analysis, the correlation coefficient (r), intercept, slope and relative standard deviation values were found satisfactory for the proposed chemometric techniques in Table 3. As can be seen, all the statistic values indicated that all techniques are convenient for the determination of two drugs in synthetic mixtures.

A summary of the assay results for the pharmaceutical formulation is given in Table 4. The results of all methods were very similar to each other as well as to the label value of commercial drug formulation.

Table 4

Assay results for the pharmaceutical formulation (mg/tablet)

Drug	CLS	ILS	PLS
LEVO Mean ± SD*	498.4± 1.12	497.1± 1.18	499.4±1.75
NORF Mean ± SD*	397.3±3.84	398.2±3.70	399.2±3.42

Results obtained are average of six experiments for each technique.

*SD : Standard deviation

CONCLUSION

Three chemometric techniques in spectrometric analysis, CLS, ILS and PLS, were proposed for the simultaneous determination of LEVO and NORF in their binary mixtures. These techniques were applied with great success to two commercial pharmaceutical tablets. The resolution of highly overlapping drug mixtures was achieved by the use of CLS, ILS and PLS techniques. A selection of working wavelengths was found having high correlation values with concentration due to interference coming from matrix sample or additional analytes outside the working range. The proposed chemometric techniques can be applied for the routine analysis of two drugs in the tablet formulation without any *a priori* chemical separation and without being time consuming.

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