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Effect of genetic algorithm as a variable selection method on different chemometric models applied for the analysis of binary mixture of amoxicillin and flucloxacillin: A comparative study

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ABSTRACT

Different chemometric models were applied for the quantitative analysis of amoxicillin (AMX), and flucloxacillin (FLX) in their binary mixtures, namely, partial least squares (PLS), spectral residual augmented classical least squares (SRACLS), concentration residual augmented classical least squares (CRACLS) and artificial neural networks (ANNs). All methods were applied with and without variable selection procedure (genetic algorithm GA). The methods were used for the quantitative analysis of the drugs in laboratory prepared mixtures and real market sample via handling the UV spectral data. Robust and simpler models were obtained by applying GA. The proposed methods were found to be rapid, simple and required no preliminary separation steps.

1. Introduction

Amoxicillin (AMX), (6R)-6-[α-D-(4-Hydroxyphenyl) glycylamino] penicillanic acid Fig. 1(a), is one of the most frequently used β-lactam antibiotics in the world. It is employed to treat humans and animals [1–3]. Various analytical methods have been reported for the separation and determination of AMX based on spectrophotometry [4–13], capillary electrophoresis [14,15], high-performance liquid chromatography [16–18] and electrochemical techniques [19–22].

Flucloxacillin (FLX), (6R)-6-[3-(2-Chloro-6-fluorophenyl)-5-methylisoxazole-carboxamido] penicillanic acid Fig. 1(b), is a bactericidal agent used primarily for the treatment of infections due to staphylococci resistant to benzyl penicillin [1–3]. There are several methods available in the literature for the quantification of FLX including high-performance liquid chromatography [23–28], spectrophotometry [4,29–32], IR and Raman spectrometry [33], polarography [34] and potentiometry [35].

Few methods were reported for simultaneous determination of both components including high performance liquid chromatography [36–38], spectrophotometry [39,40] and traditional chemometric assisted techniques [40].

Chemometrics has found its place in chemistry for about 40 years since it was established in its modern form [41]. Using its valuable tools, chemists are able to simplify or interpret complex chemical information from an often dynamic unstable system. Multivariate calibration is one of the main focus areas in chemometrics [42]. Multivariate calibration allows all measured information to be incorporated in a model. This leads to a number of advantages, for example, modeling an analyte of interest in the presence of interferences or handling noisy data [43]. It is shown that elimination of uninformative or irrelevant variables improves the model performance both theoretically [44] and experimentally [45]. As a result, different variable selection methods have been considerably developed or evaluated in order to find relevant variables or eliminate irrelevant ones [46]. In the present work, the effect of variable selection (genetic algorithm GA) [47–50] on different chemometric models was studied along with raw data models. These models which are partial least squares (PLS-1) [42,51–54], spectral residual augmented classical least squares (SRACLS) [55], concentration residual augmented classical least squares (CRACLS) [56] and artificial neural networks (ANNs) [57–59] were applied for the simultaneous determination of AMX and FLX in their binary mixtures and pharmaceutical dosage forms.

2. Experimental

2.1. Materials and reagents

A. Amoxicillin (99.84%) and Flucloxacillin (99.73%); kindly supplied by EIPICO pharmaceutical Company, Egypt.

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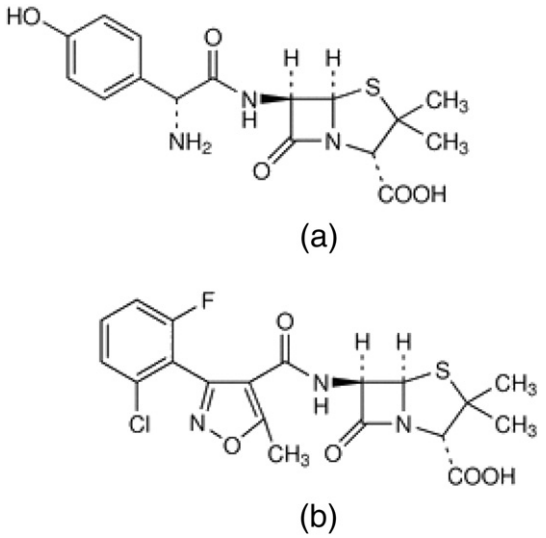

Fig. 1. Chemical structure of (a) amoxicillin and (b) flucloxacillin.

B. Flumox® Capsules dosage forms; labeled to contain 250 (AMX)/250 (FLX) mg batch number 1405340, manufactured by EIPICO Pharmaceuticals Company.
C. Sodium hydroxide; El-NASR Pharmaceutical Chemicals Co., Egypt.
D. Distilled water

2.2. Instruments

SHIMADZU dual beam UV–visible spectrophotometer (Kyoto/Japan), model UV-1800 PC connected to IBM compatible and an HP1020 laser jet printer. The bundled software, UV-Probe personal spectroscopy software version 2.43 (SHIMADZU) was used. The spectral band was 2 nm and scanning speed is 2800 nm/min with 1 nm interval.

2.3. Software

All chemometric methods were implemented in Matlab 8.2.0.701 (R2013b). PLS, SRACLS and CRACLS were performed using our own written codes in Matlab. GA and ANN were performed using PLS toolbox software version 2.1 in conjunction with Neural Network toolbox. The *t*-test and *F*-test were performed using Microsoft® Excel. One way ANOVA test was performed using Graph Pad Prism version 5 (Graph Pad, San Diego, CA).

3. Procedures

3.1. Standard solutions

Standard working solutions for AMX and FLX 100 μg mL^{-1} were prepared by dissolving the appropriate amount in 0.1 N NaOH.

3.2. Spectral characteristics of AMX and FLX

The zero-order (D_0) absorption spectra of AMX and FLX solutions (20 μg mL^{-1} for each) were recorded against 0.1 N NaOH as a blank over a range of 200–400 nm. The wavelengths used were in the range of 215–315 nm for AMX and FLX. Wavelengths less than 215 nm were rejected due to the noisy content. Wavelengths more than 315 nm were not used because the studied compounds do not absorb in this region.

3.3. Experimental design for chemometric methods

A 5-level, 2-factor design was performed using 5 concentration levels for each of the 2 compounds resulting in 25 mixtures. The design spans the mixture space fairly well [60]. The central level of the design is 20 μg mL^{-1} for each of AMX and FLX. The chosen concentrations for each compound are based on its linearity and the ratio between the two compounds involved in their pharmaceutical preparation. Table 1 represents the concentration design matrix. Thirteen mixtures of this design were used as a calibration set and the other twelve mixtures were used as a validation set to test the predictability of the developed multivariate models.

Calibration data set selection based on optimality criteria improved the quality of multivariate models predictions by improving the representativeness of the calibration data. So, D-optimal selection algorithm was used for the calibration data set selection [61].

The 2D plot of the experimental space showing the positioning of the training set and the validation set samples was presented in Fig. 2.

3.4. Analysis of AMX and FLX in FLUMOX® capsules by the proposed methods

The content of eight capsules was weighed and mixed. Appropriate weight of powder equivalent to 25 mg of the components was transferred to 250 mL flask and the volume was made up to 150 mL with 0.1 N NaOH. The solution was shaken vigorously for 15 min then sonicated for 30 min and the volume was completed to 250 mL with solvent and filtered into 250 mL volumetric flask.

Necessary dilutions of the filtrate were made with 0.1 N NaOH to obtain a different concentration of the mixture. The spectra of these solutions were scanned from 200 to 400 nm, stored in the computer and analyzed by the proposed methods.

4. Results and discussion

The UV spectra of the two compounds under study show severe overlap due to structural similarity Fig. 3, which creates difficulty in the analysis of such mixture by univariate approaches of handling UV data. Therefore, multivariate calibration methods were applied to predict the concentrations of AMX and FLX in both calibration and validation sets as well as in their pharmaceutical formulation.

4.1. Variable selection method (GA)

GA searches the solution space of a function through the use of simulated evolution. It solves the optimization problem by exploring all regions of the potential solutions and exponentially exploiting promising areas through mutation, crossover, and selection operation applied to individuals in the populations. A critical issue of successful GA performance is the adjustment of GA parameters. In order to avoid the risk of overfitting, a number of independent short runs was done and the results of all the runs were taken into consideration to obtain the final model. Doing this, a much more consistent (and less overfitted) solution can be obtained [61]. The adjusted GA parameters with the lowest mean square error were shown in Table 2. The GA was run on 101 variables for AMX and FLX using a PLS with the maximum number of LVs determined by cross-validation on the model containing all the variables. The selected variables were used for running of PLS, SRACLS, and CRACLS models and as input data for ANN. GA reduced absorbance matrix to about 42–21% of the original matrix (42 and 21 variables for AMX and FLX respectively) as shown in Fig. 4. The effect of GA on these models will be discussed next.