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Original Research Article

Simultaneous Spectrofluorometric Determination of Fluoroquinolones Using Principal Component Analysis in Biological Samples

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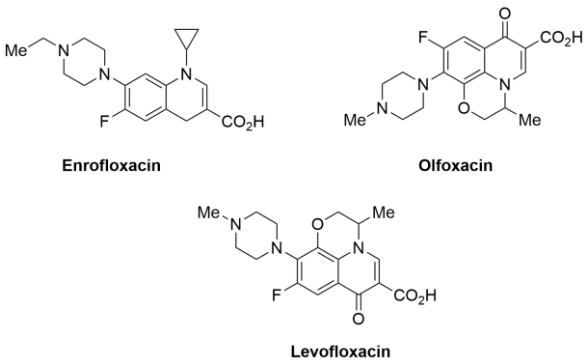
ABSTRACT

In the present work, a very sensitive, reliable, and simple spectrofluorometric procedure was improved for concurrent determination of ofloxacin, enrofloxacin, and levofloxacin in the absence of separation treads. Despite a spectral cover, a part of fluoroquinolones have been concurrently resolved by chemometric come near to involving principal component analysis artificial neural network and partial least squares. Artificial varieties mixtures of fluoroquinolones were evaluated and the results acquired by the implementations of these chemometric approaches were evaluated and compared. It was found that the principal component, artificial neural network method provided relatively better accuracy than that of PLS method. This method was applied satisfactorily for determining mixtures of fluoroquinolones in tilapia, chicken samples, and synthetic samples (with concentration ranging over 0.05-1.1 µg/mL for ofloxacin as well as 0.06-0.6 µg/mL and 0.01-0.23 µg/mL for enrofloxacin and levofloxacin), respectively. The suggested method enables detection limits of 0.04, 0.01, and 0.009 µg/mL for ofloxacin, enrofloxacin, and levofloxacin, correspondingly. The recoveries in the tilapia and chicken matrices ranged from 114% to 92%. All experiments that needed to be repeated were repeated 4 times.

GRAPHICAL ABSTRACT



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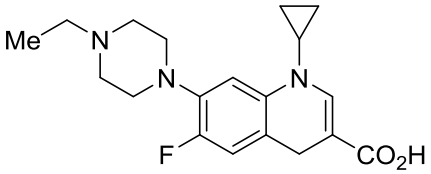
Introduction

Introduction Fluoroquinolones (FQs) are piperazinyl derivatives of quinolones (Scheme 1), and are routinely applied in human and veterinarian drug as antibacterial factors versus some diseases over the recent decade [1, 2]. The attendance of FQs in edible animal result of manufacturing is a meaningful risk that can be clearly deadly or cause of pathogen opposition in humans [3]. Therefore, selective and sensitive analytical procedures are needed for quick detection and recognition of FQs in the environmental and biological products. Various chromatographic and spectrophotometric methods [4-9] have been discovered for detection of FQS in different samples. Amid these ways spectroflorometric methods due to the upward fluorescence exhibited by most of the FQS was reasonable appropriate for its sensitivity and specificity [10]. Fluorescence detection is highly selective because both emission and excitation wavelength maximum are attainable to describe a definite compound and due to the fact that only a restricted quantity of compounds fluoresce [11]. In addition, spectrofluorometric is simpler, inexpensive, and more available than modern and expensive apparatus such as HPLC, LC-mass, and GC-mass that are not available. Therefore, over the concurrent determination of quinolones by the use of the customary spectrophotometry or spectrofluorometric caused an extent over of their emission and absorption spectra and matrix effects is difficult absence separation.

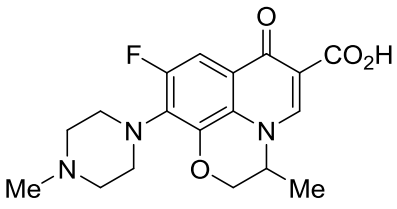
However, the use of various multivariate techniques can avoid these problems without the need for a previous separation step. Recently, quantitative spectrofluorometry has been greatly improved by the use of statistical methods, especially PLS and ANN. In recent years, ANN has been regarded as a famous way in fashioning complex non-linear systems and providing extract accurate data from individual measurements [12, 13].

The theory and diligence of ANN and PLS in spectrometry have been improved by various researchers [14-19]. At the same time, several multi-component determinations of inorganic substances established on, the diligence of these methods to spectrometric data have been provide [20-24].

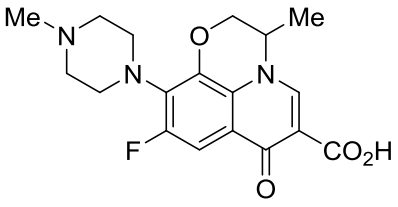
The main aim of those reports is improving a simple and proper method for simultaneous spectrofluometric monitoring of ofloxacin, enrofloxacin, and levofloxacin traces in different real samples by chemometric approximations like PLS and PC-ANN. In the PC-ANN template, several principal component analyses (PCA) of the reply data of the calibration data were utilized as an input data.



Enrofloxacin



Olfoxacin



Levofloxacin

Scheme 1: Chemical structure of ofloxacin, enrofloxacin, and levofloxacin

PC-ANN arrangements were assembled using distinctive numbers of PCs. A hidden layer was composed of neurons with sigmoid operation. The general equally balanced state for the discipline of weights with momentum was utilized to decline the time for assembly, and also to avert networks from developing trapped in area minimum [25]. The multilayer act of supplying forward PC-ANN was inculcated by a back-propagation step-by-step procedure used to solve a problem. The variables of PC-ANN formation were improved to acquire the minimum mistake for the forecasting set. On the other hand, for concurrent determination of ofloxacin, enrofloxacin, and levofloxacin by PLS, the sum of principal components were improved efficiency and at the improve number of PC, the concentrations of the set were acquired. The consequences acquired by two ways are shown how are alike or different and discussed.

Materials and Methods

Apparatus

The fluorescence spectrophotometer utilized in this work was a Cary Eclipse (Varian-Australia) including a photomultiplier tube detector, which was arranged at the wavelength from 190-800 nm. Cell with 1 cm optical path length and bandwidths were arranged 10 nm utilized. The inscribed spectra converted analog data into digital data with an intermission of 1 nm between sequential. Therefore, 230 data set points were utilized to symbolize a spectrum in the interval of 300-530 nm. The ultrasonic processor device model UP-100H (Hielscher-Germany) was utilized in this research. PH evaluations were achieved with a Metrohm 780 pH-meter (Herisau). The calculations were down on a pentium 4,2.4 GHz computer. All the plans were registered in MATLAB (version 7).

Reagents and materials

Ofloxacin (OFL), enrofloxacin (ENR) and levofloxacin (LEV) were provided rom Razi Company (Tehran, Iran). Methanol (MeOH), hydrochloric acid HCl (37% w/w), acetonitrile (ACN), and metaphosphoric acid of ultra-high grade were obtained from Merck (Darmstadt, Germany). Fresh working solutions were prepared daily by diluting the stock solution in distilled water. Stock working solutions (10 mg/mL) were prepared by dissolving calculated amounts of them in methanol. Worked solution of each quinolone (100 µg/mL) was made ready in methanol. In addition, worked solutions were made day by day inward diluting the solution by deionized water.

Real samples preparation

The tilapia fish samples were obtained from the local forums in Mashhad, Iran, and every one 1.0 g of homogenized specimens was take out with 2.0 mL acetonitrile–acetic acid (95: 5 V/V) for 10.0 min by ultrasonic processor. The supernatants were combined by centrifugation at 5000 rpm and concentrated to lack of moisture at 50°C, and at the same time reconstituted by methanol for subsequent steps [26, 27].

The chicken samples were obtained from the local forums in Mashhad, Iran, and then sample was initially shaped by grinding and 1.0 g of tissue was burdened, followed which 2.0 mL of 0.3%methaphosphoricacid-acetonitrile (1:1V/V) solution was added with ultrasonic-assisted for 10 min. The extraction was expressed again for three times. The supernatants were gathered, followed by centrifugation to separate at 6000 round per minute for10 min. The solutions become dry at 50 °C and finally 1.0 mL of methanol was added for further steps [28, 29].

Fluorescence emission spectra measurement

The samples were prepared with appropriate volumes of standard solutions each quinolones and by adding drop wise HCl (0.1M) to adjust pH 4.0 to a 10 mL volumetric flask and fabricated to the mark with pure water to acquired definitive concentration in the interval of 0.01-0.9 µg/mL. At each experimental, about 0.7 mL of the above solution was conveyed to a cell and the fluorescence emission spectrum was listed from 300-530 nm, using an excitation of 278 nm.

Results and Discussion

Introductory researches exhibited that ofloxacin, enrofloxacin, and levofloxacin show inherent fluorescence in acid mediocre, with greatest signals having emission and excitation spectra of 270 and 485 nm for ofloxacin, 275 and 445 nm for enrofloxacin, and 290 and 420 nm for levofloxacin, correspondingly. Therefore, excitation wavelength 278 nm was picked out for investigating mixtures of these quinolones. Figure 1 displays the emission spectra for each quinolones and for composition beneath this condition in water and Figure 2 depicts the role of the pH in emission strength for three quinolones exploiting a 278 nm excitation wavelength. It is well-known that the pH of donor solutions plays an essential role in the extraction of basic drugs. The quinolones exhibited a steadfast emission in comparable pH range 3-4. Based on comprehensive consideration, pH 4 was selected to investigate mixtures of these quinolones.

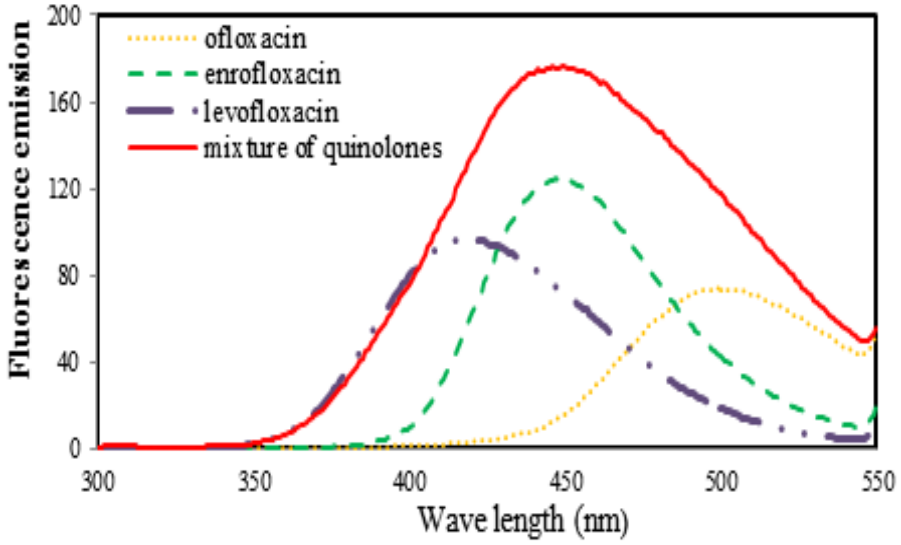


Figure 1: Fluorescence emission spectra obtained for 1 µg/ $mL^{-1}$of the three quinolones in water

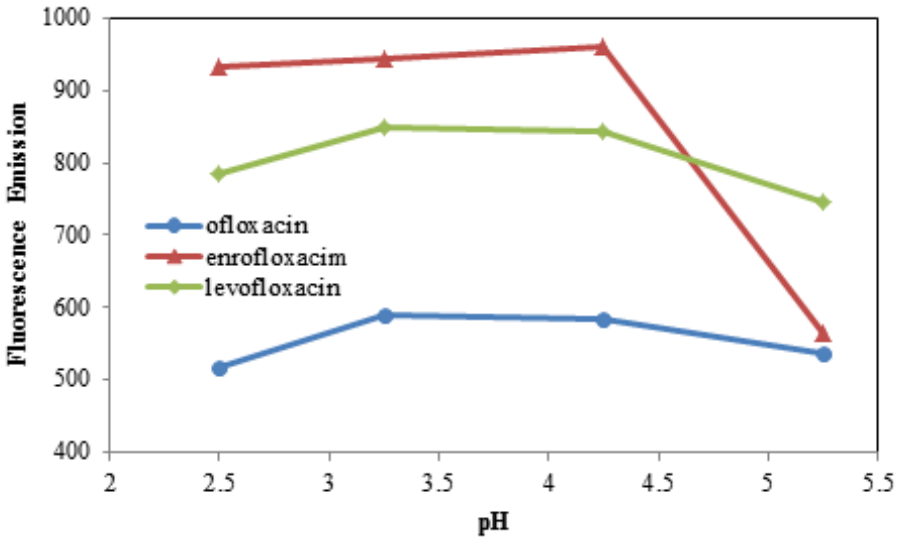


Figure 2: Effect of the pH in fluorescence emission of ofloxacin, enrofloxacin, and levofloxacin

Chemometric studies

The standard solutions of OFL, ENR, and LEV were prepared by precise dilutions of corresponding stock solutions with distinctive concentrations in their dynamic ranges. Then, 21 working solutions were randomly selected for the training arranged (Table 1), 7 solutions for the prediction, and 7 solutions for the validation set. To comparison the consequences of the two patterns, the prediction sets were exhibited similarly for two models. In each case, the correlation between amount concentrations of compounds was prevented because co-linear components in the training data care for to cause over fitting in these patterns. The spectrum for each of cares was listed from 300 to 530 nm. Hence, for example, the response information of the training set was a matrix with 21×231 size. At the beginning, in PC-ANN the matrix of the training set was examined by PCA and PCs scores were selected as input. Three sheet back-propagation networks were utilized with learning speed (λ=0.4, 0.5, 0.5 for OFL, ENR, and LE, respectively) and momentum (μ=0.6, 0.9, 0.3 for OFL, ENR, and LEV, respectively). The variables of network layout were composed of PCs as an input; the number of nodes in the hidden stratum and the number of epochs were improved efficiency. It should be well-known that the actuality amount of PCs and nodes in the hidden stratum are chosen on the basis of the smallest amount for the mean square errors for prediction (MSEP). In improve efficiency the number of PCs and the number of nodes hidden stratum, PC-ANN layout with distinctive numbers of PCs and distinctive numbers of nodes in the hidden stratum were researched with 1×10^4 period. The mean square errors of prediction (MSEP) at each of the layouts were estimated. The consequences inhibited the MSEP is lower with 8, 6, and 6 PCs for OFL, ENR, and LEV, correspondingly and 5, 5, and 6 knots in the hidden layer for OFL, ENR, and LEV correspondingly. Since 1.5×10^5, 2×10^5, and 1.7×10^5 repetition, MSEP declined extremely slowly for OFL, ENR, and LEV. Hence, the number of 1.5×10^5, 2×10^5, and 1.7×10^5 epochs were chosen for OFL, ENR, and LEV, correspondingly.

In PLS model, the model of cross-validation for the calibration set was utilized to determine the amount of principal components [30, 31]. Figure 3 is a scheme of prediction residual sum of squares (PRESS) versus the number of causes (nc). One plausible selection for the optimum number of factors would be that number which produced the minimum PRESS. However, using the number of causes (h*) that producing a smallest press commonly lead to some fitting. A better scale for choosing the best number of factors including the likeness of PRESS from patterns with less from h* factors. Hal and Over Thomas experimentally determined that an F-ratio reasonability of 0.75 is a good selection [12]. Anyway, we chose it as the best number of agents for the PRESS amount the F-ratio reasonability which make fall under 0.75. The greatest number of agents to estimate the best PRESS was 10, and the number of factors acquired by the application of PLS pattern was 5, 3, and 4 forofloxacin, enrofloxacin, and levofloxacin, respectively. The effect achieved by implementing PC-ANN and PLS to the prediction series are presented in Table 1. The tabulated consequences in Table 1 also exhibited the retrieval for prediction consequences for a series of fluoroquinolones mixtures. Suitable recovery amount are performed in specimens analyzed in the prediction series by the two methods in greater concentrations; but in lessen concentrations, the consequences of PC-ANN were considerably better from PLS. For the assembled model, three general statistical factors were chosen to estimate the prediction capability of the model for concurrent determination of fluoroquinolones. The initial statistical factor is the root mean square difference (RMSD). This factor is a statement of the intermediate error in the analysis at each component. RMSD was achieved by the subsequent equation:

$$RMSD = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(\hat{x}_i - x_i)^2}$$

Table 1: Simultaneous determination of OFL, ENR and LEV in the prediction set[a]

PLS									PC-ANN					
			Amount found			Recovery%			Amount found			Recovery%		
OFL	ENR	LEV	OFL	ENR	LEV	OFL	ENR	LEV	OFL	ENR	LEV	OFL	ENR	LEV
0.05	0.17	0.65	0.04	0.21	0.65	80	123.5	100	0.05	0.16	0.66	100	94.1	101.5
0.89	0.23	0.01	0.73	0.26	0.01	82	113	100	0.84	0.24	0.01	94.3	104.3	100
0.28	0.06	0.96	0.25	0.10	0.88	89.2	166.6	91.6	0.29	0.07	0.90	103.5	116.6	93.7
0.47	0.23	0.49	0.37	0.24	0.50	104.2	104.3	102	0.41	0.23	0.53	87.2	100	108.1
0.51	0.4	0.5	0.50	0.40	0.49	98	100	98	0.53	0.42	0.53	103.9	105	106
0.1	0.5	0.6	0.11	0.49	0.49	110	98	81.6	0.11	0.48	0.57	110	96	95
0.4	0.6	0.2	0.38	0.54	0.20	95	90	100	0.43	0.58	0.18	107.5	96.6	90

[a]*All the concentrations are as mg/L.*

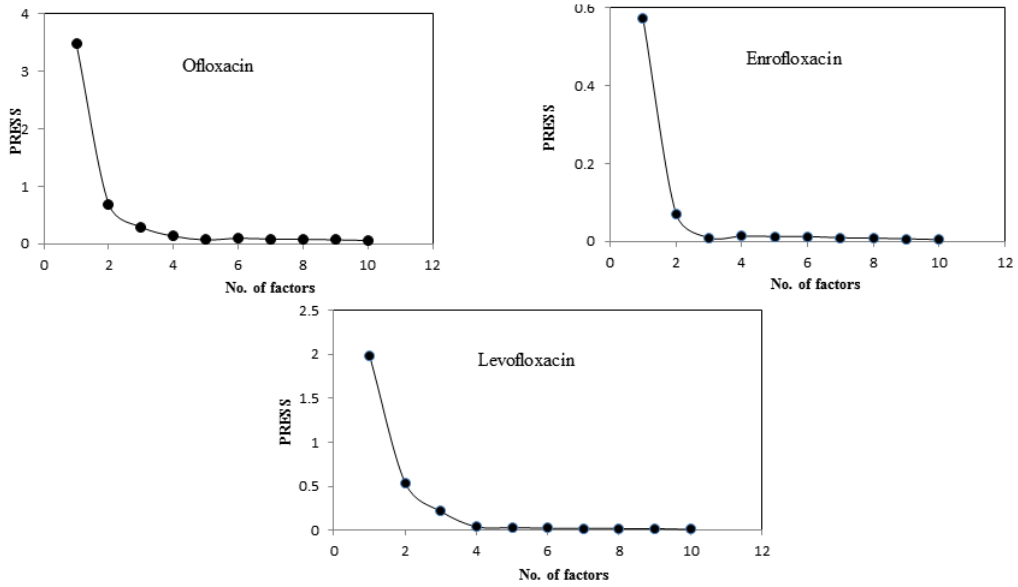


Figure 3: Cumulative PRESS as a function of number of factors for PLS method

The second statistical factor was relative error at prediction (REP) which displays the forecasting capacity of every component and is estimated as:

$$REP = \frac{1}{\bar{x}}\sqrt{\frac{1}{n}\sum_{i=1}^{n}(\hat{x}_i - x_i)^2}$$

In the mentioned equations, $\boldsymbol{x_i}$ is the actual concentration in the specimen i, $\hat{x}_i$ the forecasted concentration of the by the method in the sample i, $\bar{x}$ is the mean of true concentration in the prediction series and n is the entire number of samples utilized in the prediction. The statistical consequences (RMSD, REP, and R^2) for validation, prediction, calibration, and sets are summarized in Table 2. The consequences of this table show the prosperous ability to be implemented of the PC-ANN pattern for concurrent determination of fluoroquinolones at real specimen so that estimate the execution of the PC-ANN template for concurrent determination of fluoroquinolones another set as validation series was applied. Thereafter, the calculated concentrations versus anticipated concentrations were plotted for each quinolones. The schemes are presented in Figure 4 for ofloxacin, enrofloxacin, and levofloxacin, respectively. The correlation coefficient (R^2), that

reveals the characteristic of suitability of all the data to a without delay line, is estimated for examine of every calibration. The suitable correlation of coefficient (0.990 for ofloxacin, 0.997 forenrofloxacin, and 0.995 for levofloxacin) shows the ability of the PC-ANN template for concurrent determination of ofloxacin, enrofloxacin, and levofloxacin.

Table 2: Statistical parameters of the PC-ANN and PLS methods for calibration, validation, and prediction sets at optimum significant factors

Set	Component	PLS			PC-ANN		
		RMSD[a]	REP[b] (%)	R^{2c}	RMSD[a]	REP[b] (%)	R^{2c}
Calibration set	OFL	0.059	13.107	0.970	0.026	5.596	0.990
	ENR	0.023	7.515	0.986	0.012	3.937	0.997
	LEV	0.033	9.136	0.985	0.018	5.639	0.995
Validation set	OFL	-	-	-	0.023	5.917	0.995
	ENR	-	-	-	0.018	5.614	0.984
	LEV	-	-	-	0.032	6.406	0.992
Prediction set	OFL	0.073	18.890	0.979	0.033	8.543	0.987
	ENR	0.034	10.737	0.994	0.015	4.679	0.996
	LEV	0.052	10.610	0.983	0.033	6.719	0.988

a :Root mean square difference.
b :Relative error of prediction.
c :Correlation coefficient.

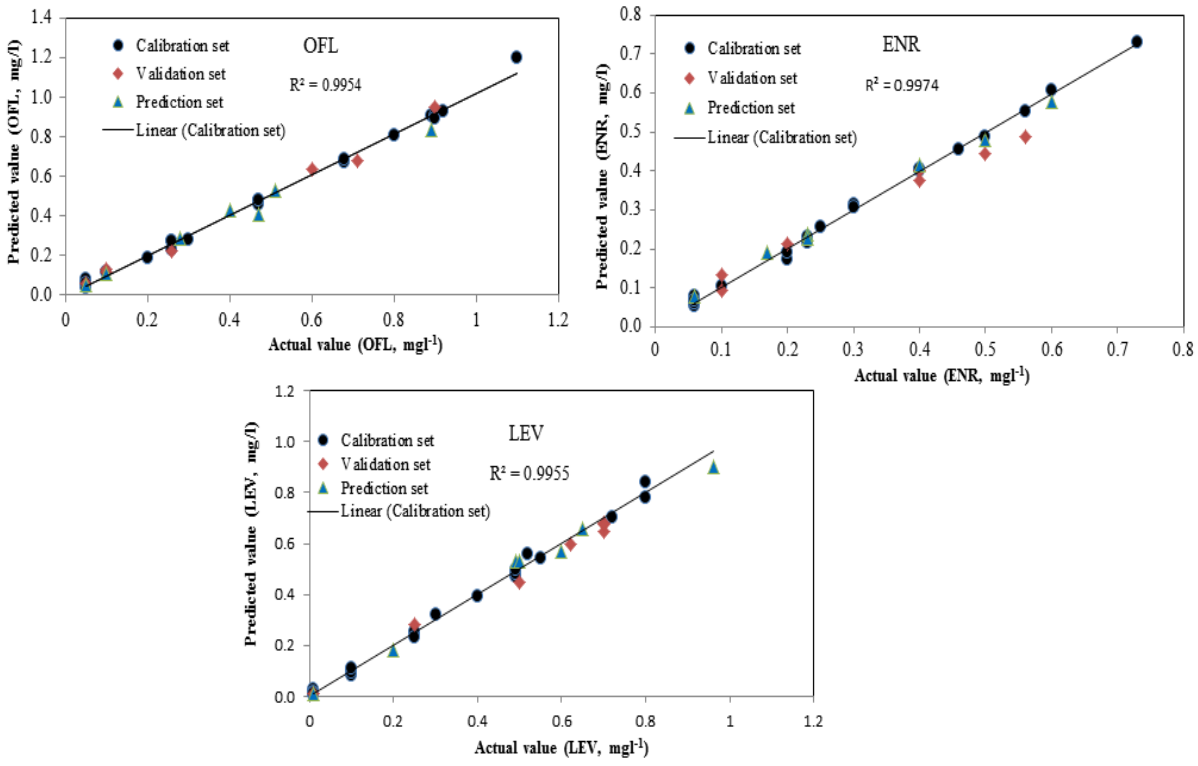


Figure 4: Relationship between expected concentration of OFL, ENR, and LEV against the calculation of OFL, ENR, and LEV determined by PC-ANN for the calibration, validation, and prediction sets

Analysis of the real samples

Examination of the real specimen gathered from the vernacular markets of Mashhad was utilized for the validation of the suggested PLS and PC-

ANN. The recovery research was also behaved to scrutinize the influence of the sample matrix by addition three different amounts of target analytes in the tilapia and chicken samples. The consequences revealed that (PC-ANN) model was in a prosperous manner useable for determination of ofloxacin, enrofloxacin, and levofloxacin in sample. Correspondingly, in all occasions the recoveries disclose the ability of this method for concurrent determination of these compounds (Table 3).

Table 3: Determination of OFL, ENR, and LEV in real sample (PC-ANN method)[a]

Sample	Added			Found			Recovery%		
	OFL	ENR	LEV	OFL	ENR	LEV	OFL	ENR	LEV
Fish	0.85	0.20	0.05	0.82	0.19	0.048	96.47	95.00	96.00
	0.22	0.07	0.49	0.25	0.08	0.51	113.64	114.29	104.08
	0.10	0.33	0.20	0.11	0.31	0.19	110.00	93.94	95.00
	0.08	0.15	0.35	0.09	0.14	0.38	112.50	93.33	108.57
Chicken	0.45	0.55	0.15	0.48	0.52	0.17	106.67	94.55	113.33
	0.10	0.20	0.25	0.095	0.22	0.23	95.00	110.00	92.00
	0.30	0.25	0.09	0.28	0.27	0.10	93.33	108.00	111.11

[a] *All the concentrations are as mg/L.*

Conclusion

A new aim for the concurrent determination of ofloxacin, enrofloxacin, and levofloxacin mixture with the aim of multivariate calibration aim (PLS) and PC-ANN was recommended, because of the wide spectral having something in common observed between the emission spectra of their constituent. In two procedures, the principal components were utilized, but in PC-ANN achieved responses were excellent and pleasant. In fact, achieving of PC-ANN to spectrofluometric data enables concurrent determination of these three quinolones with excellent precision, selectivity, sensitivity, and a large domain of linearity. This research was utilized determination of these target analytes in actual and synthetic samples with gratifying consequences.

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Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

We have no conflicts of interest to disclose.

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