Iranian Journal of Pharmaceutical Sciences
2014: 10 (3): 19- 34
www.ijps.ir

Original Article

Adaptive Neuro-Fuzzy Inference System (ANFIS) Applied for Spectrophotometric Determination of Fluoxetine and Sertraline in Pharmaceutical Formulations and Biological Fluid

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Abstract

The UV-spectrophotometric method of analysis was proposed for simultaneous determination of fluoxetine (FLX) and sertraline (SRT). Considering the strong spectral overlap between UV-Vis spectra of these compounds, a previous separation should be carried out in order to determine them by conventional spectrophotometric techniques. Here, full-spectrum multivariate calibrations adaptive neuro-fuzzy inference system (ANFIS) method is developed.Adaptive neuro-fuzzyinference system (ANFIS) is a neuro fuzzy technique where the fusion is made between the neural network and the fuzzy inference system that is a computational method. The experimental calibration matrix was constructed with 30 samples. The concentration ranges considered were 5-120$\mu g.mL^{-1}$fluoxetine and 10-120$\mu g.mL^{-1}$sertraline .Absorbance data of the calibration standards were taken between 200-300nm with UV-Vis spectrophotometer. The method was applied to accurately and simultaneously determine the content of pharmaceutical in several synthetic mixtures and real samples. Assaying various synthetic mixtures of the components validated the presented methods. Mean recovery values were found to be 101.26% and 100.24%, respectively for determination of FLX and SRT.

Keywords: Adaptive neuro-fuzzy inference system (ANFIS), Biological fluids, Fuzzy logic, Fluoxetine, Sertraline, Spectrophotometric.

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Cite this article as: Akbari Hasanjani H, Sohrabi M, Abdolmaleki P, Adaptive Neuro-Fuzzy Inference System(ANFIS) Applied for Spectrophotometric Determination of Fluoxetine and Sertraline in Pharmaceutical Formulations and Biological Fluid. Iranian Journal of Pharmaceutical Sciences, 2014, 10(3): 19-34.

1. Introduction

Depression is the most common mental disorder that affects a large number of individuals in all countries. However, depression is under diagnosed and frequently under treated [1]. Recent evidence suggests that depressive episodes, if left untreated, may heighten severity of subsequent episodes and

may increase need for more health care resources. The importance of depression as a major public health problem is emphasized by finding its place in the range of global burden of diseases. Depression was the fourth largest cause of disease worldwide in 1990, and by 2020 it is expected to be the second largest cause of disease [2]. This problem can become chronic or recurrent and lead to substantial impairments in an individual's ability to take care of his/her everyday responsibility. At its worst, depression can lead to suicide, a tragic fatality associated with the loss of about 850 000 lives every year [3].

Fluoxetine HCl (FLX) is an antidepressant of the selective serotonin reuptake inhibitor (SSRI) class. It is chemically designated as N-methyl-3-phenyl-3-[4-(trifluoromethyl) phenoxy]propan-1-amine, (Fig. 1A).

It is used for the treatment of depression. Being one of SSRI drugs, it acts by increasing the extracellular level of the neurotransmitter serotonin by inhibiting its reuptake into the cell [4].

Fluoxetine is used in the treatment of major depression (including pediatric depression), panic disorders and premenstrual dysphonic disorder. It has been used for cataplexy, obesity and alcohol dependence [5].

Sertraline [(1S,4S)N-methyl-4-(3,4-dichloro phenyl)- 1,2,3,4-tetrahydro-1-naphthylamine, SER] (Fig. 1B) , categorized as a second generation antidepressant drug belongs to the SSRI class. It is approved by the USFDA for the treatment of depression, obsessive–compulsive disorder, posttraumatic stress disorder, social anxiety disorder, postmenopausal dysphoric disorder, and panic disorder [6].

Several methods have been reported for the determination of fluoxetine (I) in biological fluids and pharmaceutical formulations including HPLC [7-14], GC [14-18], sertraline hydrochloride (II) determined in biological fluids and dosage forms by GC mass [18-21], GC [22] and HPLC [23-26].

UV–Vis spectrophotometric techniques are some of the mostuseful analytical methods, due to the experimental speed and simplicity, and also to their wide application range. However, the simultaneous determination of fluoxetine and sertraline by using traditional spectrophotometric techniques is difficult, because the absorption spectra usually overlap and hence they are not suitable for quantitative evaluation [27-29].

It is worth mentioning that over the past decade, significant advances have been made in two technological areas: fuzzy logic (FL) and neural networks (NNs). The synergism of FL systems and NN has produced a functional system capable of adapting its structure based on external or internal information; in other words, these systems may be compared to biological systems, in terms of learning, high-level thinking and reasoning [30]. It is an improved tool for determining the behavior of imprecisely defined complex systems. The purpose of a neuro-fuzzy system is to apply neural learning techniques to identify the parameters and/or structure of neuro-fuzzy systems. These neuro-fuzzy systems can combine the benefits of the two powerful paradigms into a single capsule. They have

several features, which make them suitable for a wide range of scientific applications. These strengths include fast and accurate learning, good generalization capabilities, excellent explanation facilities in the form of meaningful fuzzy rules, and the ability to accommodate both data and existing expert knowledge about the problem under consideration. The goal of ANFIS is to find a model or mapping that will correctly associate the inputs with the target (concentration). Fuzzy inference system (FIS) [31] is a knowledge representation where each fuzzy rule describes a local behavior of the system. The network structure that implements FIS is referred to as ANFIS and employs hybrid learning rules to train a Sugeno-style FIS [32] with linear rule outputs. However in this work we have used ANFIS to train a fuzzy inference system that applied to simultaneous determination of sertraline and fluoxetine hydrochloride in several synthetic mixtures.

2. Material and Methods

2.1. Apparatus and Software

A Shimadzu uv-2100 double-beam UV–Vis spectrophotometer with a 1.0 cm path length quartz cell was utilized. Calculations and the signal transforms were performed in EXCEL and MATLAB 7.1 environment. The spectrophotometric measurements were carried out at room temperature (mean of about 20 ℃) and all solutions were prepared on the same day before that of the analysis.

A model pHs-3C pH meter was calibrated by using buffer solutions and then used for pH measurements. The pH of the solutions was surveyed in the range of pH 2.0 – 9.0.

2.2. Reagent and chemicals

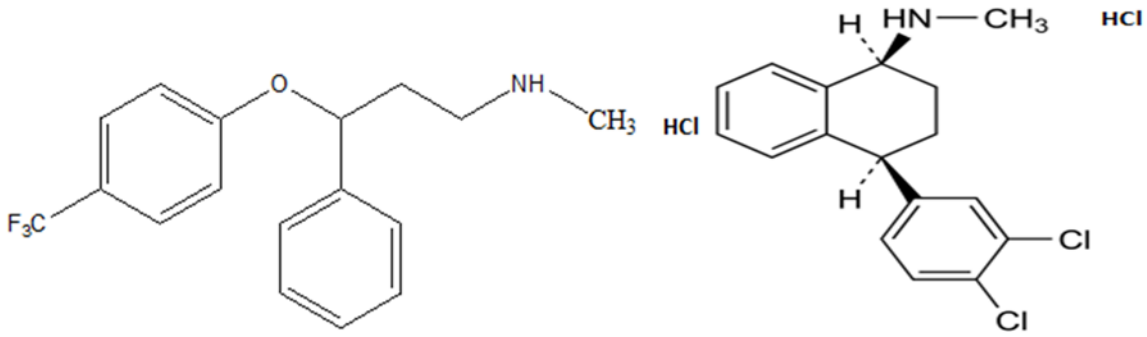
All chemicals and solvents were of analytical reagent grade. Pure Fluoxetine and Sertraline drugs and its pharmaceutical dosage form containing 20 mg of FLX and 100mg of SRT were donated by Dr. Abidi Company (Tehran – Iran). Sulfuric acid and NaOH were purchased from Merck (Darmstadt, Germany) Company.

2.3. Standard Solutions

Stock standard solutions of fluoxetine and sertraline were prepared by dissolving 20.0 mg of fluoxetine and 20.0 mg of sertraline in ethanol in 100 mL volumetric flaks and diluted to the mark with the solvent. Working solutions were prepared by appropriate dilution of the stock solution in ethanol to reach concentration ranges of 5-120$\mu g.mL^{-1}$ and 10-120$\mu g.mL^{-1}$for fluoxetine and sertraline, respectively.

2.4. Preparation of Real Samples

Ten tablets were finely powdered and an appropriate portion (equivalent to the median mass of one tablet) was dissolved in 100 ml of ethanol, It was mechanically shaken for a period of 20 min and filtered into a 250 ml calibrated flask. The residue was washed twice with the same solvent and diluted to the volume. After filtration, the obtained clear solution was adjusted to the volume of 100ml with the same solvent. This solution was



(A) (B)

Figure 1. The Structures of Fluoxetine HCl (A) and Sertraline HCl (B).

further diluted to get the suitable concentration for the UV measurements [36].

2.5. Analysis of Urine Samples

Urine spiked with fluoxetine and sertraline was obtained by following procedure; an aliquot of pure fluoxetine and sertraline was added into 10 ml urine sample.1 ml of the resulting urine solution was mixed with 5ml (0.2 M) sodium carbonate buffer and 10 ml butyl chloride. The mixture was rotated for 20 min and centrifuged at 2500 rpm for 10 min. The butyl chloride layer was separated and then evaporated till dryness. Resultant residue was dissolved in universal buffer (at different pH) into a 10 ml volumetric flask and diluted to the mark with buffer solution.

2.6. Fuzzy Logic

Fuzzy logic is used to simulate theories and perceptions of users [33] to control a fuzzy system for which a precise mathematical model where high accuracy is not required. In fuzzy logic and fuzzy controllers, the variables are offered as fuzzy sets in which lingual variables are observed. Lingual variables used to control the system are “small”, “very small”, “big”, and “very big”.

There is a strong relationship between bully logic and classic set theory and a strong relationship between fuzzy logic and fuzzy sets. Fuzzy logic was theoretical until 1970, when computers developed to the point where the first fuzzy system were developed.

A classic controller concerns accuracy and mathematical equations and a fuzzy controller

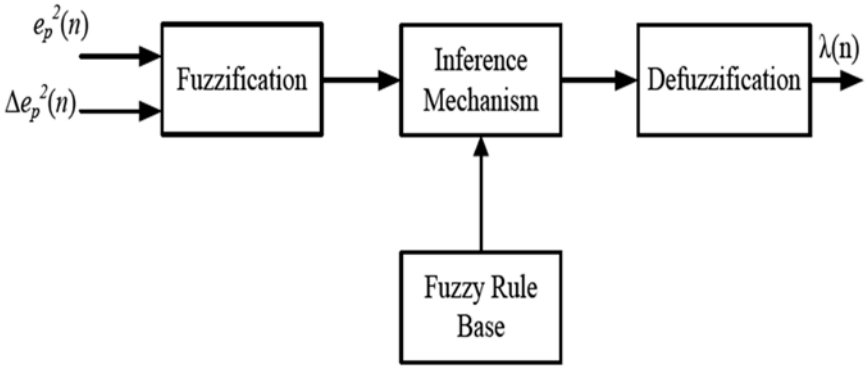


Figure 2. Structure of Fuzzy Controller.

concerns experiences, knowledge and lingual definitions of an operator [33]. No precise mathematical model is required in fuzzy control; however, if user and designer knowledge about a control process is insufficient, no satisfactory results can be obtained. Fuzzy input and a series of rules for control based on designer knowledge and experiences are required based on the structure of a fuzzy system.

Fig. 2 shows the structure of a fuzzy controller. In this controller, the input must be fuzzied to convert classic numbers to fuzzy numbers. This requires a series of fuzzy rules based on user experience. When fuzzy output is calculated based on these rules, the numbers have a fuzzy nature and must be converted to non-fuzzy numbers to be identified by other systems.

the knowledge representation of fuzzy logic results in the development of adaptive neuro-fuzzy inference systems (ANFIS) [34].

The neuro-fuzzy model in ANFIS is based on the Takagi– Sugeno-type fuzzy inference system with a single output. In this model, the output of each rule is calculated by a linear combination of the input parameters and an additional constant term. The structure of an ANFIS with two inputs (x and y) and one output (z) is shown in Fig. 3.

By assuming a first-order Sugeno fuzzy model, a common rule set with two fuzzy if-then rules for a system with two inputs and one output is the following:

Eq 1 Rule1: if x is A_1 and y is B_1 then $z_1=p_1x + q_1y + r_1$

Eq 2 Rule2: if x is A_2 and y is B_2 then $z_2 = p_2x + q_2y + r_2$

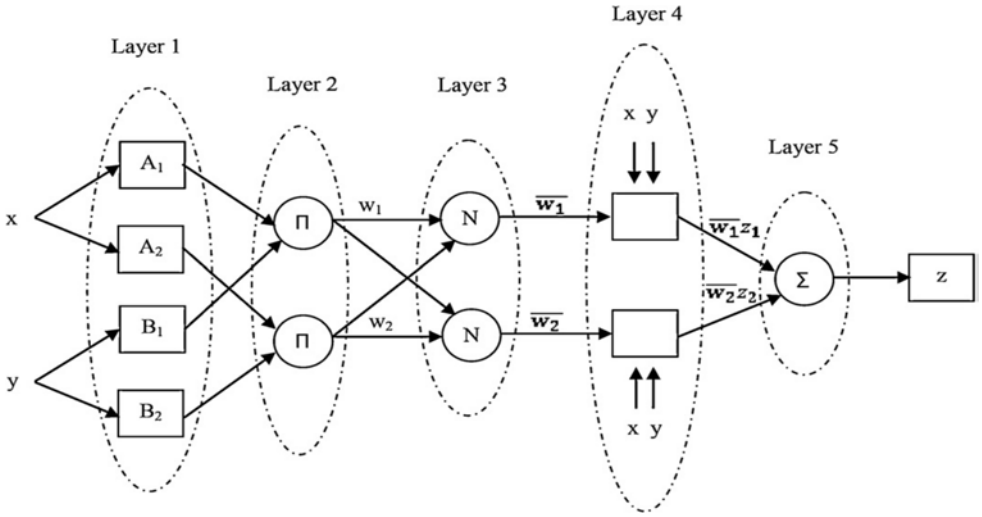


Figure 3. Adaptive Neuro-Fuzzy Inference System (ANFIS) Structure.

2.7. ANFIS Architecture

The neuro-fuzzy technology combines ANN and fuzzy logic. The combination of the learning capabilities of neural networks with

As shown in Fig. 3, the neuro-fuzzy system has a total of five layers and the nodes of each layer have the following functions: Layer 1:

every node in this layer is an adaptive node with the following node function:

Eq 3 $O_{1,i} = \mu A_i(x) \, for i = 1,2$ or

Eq 4 $O_{i,1} = \mu B_{i-2}(\mathrm{y})$, $\mathrm{i} = 3,4$

Where x and y are the inputs to the first node and A_i and B_{i-2} are the fuzzy set that is associated with this node. A ‘‘fuzzy set’’ is a simple extension of the definition of a classical set, in which the characteristic function is permitted to have any value between 0 and 1 [35]. A ‘‘fuzzy set’’ A of X can be defined as a set of ordered pairs:

Eq5 $A = \{(\mathrm{x}, \mu_A(x)) | x \in \mathrm{X}\}$

Where mA(x), which is a membership function for the fuzzy set A, maps each x to a membership grade between 0 and 1. The first step in the development of the fuzzy inference system is the determination of the optimal type and number of membership functions. The most widely used membership functions include the triangular, Gaussian, generalised bell and trapezoidal functions, which are defined as follows:

Eq6

$$\mathrm{triangle}(\mathrm{x}; \mathrm{a}, \mathrm{b}, \mathrm{c}) = \max(\min(\frac{x-a}{b-a} - \frac{c-x}{c-b}), 0)$$

Eq 7 $$\mathrm{Gaussian}(\mathrm{x}; \sigma, \mathrm{c}) = \exp(-\frac{(\mathrm{x}-\mathrm{c})^2}{2\sigma^2})$$

Eq 8 $$\mathrm{bell}(\mathrm{x}; \mathrm{a}, \mathrm{b}, \mathrm{c}) = (\frac{1}{1 + \left| x - c \middle/ a \right|^{2b}})$$

Eq 9

$$\mathrm{trapozeid}(\mathrm{x}; \mathrm{a}, \mathrm{b}, \mathrm{c}, \mathrm{d}) = \max(\min(\frac{x-a}{b-a}, 1, \frac{d-x}{d-c}), 0)$$

Where a, b, c, d, and σ are the premise parameters of the membership functions.

Layer 2: Every node in this layer is a fixed node that is labelled as II. The outputs of these nodes are the products of all of the incoming signals and represent the firing strength of a fuzzy rule:

Eq 10

$$\mathrm{O}_{2,i} = w_i = \mu A_i(\mathrm{x}) * \mu B_i(\mathrm{y}) \quad , \mathrm{i} = 1,2$$

Layer 3: Every node in this layer is a fixed node labelled N. The ith node calculates the ratio of theith rules firing strength to the sum of rules firing strengths:

Eq 11 $$\mathrm{O}_{3,i} = \overline{w_i} = \frac{w_i}{w_1 + w_2'} \quad , \; i = 1,2$$

The outputs of this layer are called the ‘‘normalised firing strengths’’.

Layer 4: Every node (i) in this layer is an adaptive node with a node function that is defined as the following:

Eq 12

$$O_{4,\mathrm{i}} = \overline{w_i} z_i = \overline{w_i} (\mathrm{p}_i \mathrm{x} + \mathrm{q}_i \mathrm{y} + \mathrm{r}_i) \quad , \mathrm{i} = 1,2$$

Where w_i is a normalised firing strength from layer 3 and $\{p_i, q_i, r_i\}$ is the parameter set of the node. The parameters in this layer are called ‘‘consequent parameters’’.

Layer 5: The single node in this layer is a fixed node that is labelled ∑, which calculates the overall output as the summation of all of the incoming signals:

Eq 13 $$O_{5,i} = \sum_i \overline{w_i} z_i = \frac{\sum_i w_i z_i}{\sum_i w_i}$$

Thus an adaptive network, which is functionally equivalent to the Takagi–Sugeno-type fuzzy inference system, was constructed. Based on the ANFIS structure described, the output z can be defined as the following:

Eq 14 $$z = \frac{w_1}{w_1 + w_2} z_1 + \frac{w_2}{w_1 + w_2} z_2 = \overline{w_1}(p_1 x + q_1 y + r_1) + \overline{w_2}(p_2 x + q_2 y + r_2)$$

Where p_1, p_2, q_1, q_2, r_1 and r_2 are the linear consequent parameters. A hybrid learning procedure was used to train the proposed ANFIS model, which includes the tuning of the premise parameters using a back propagation technique and the learning of the consequence parameters by the least-squares method. This hybrid learning algorithm is composed of two phases. The first is a forward pass in which the node outputs are passed forward until these reach layer 4 and the consequence parameters are calculated through least squares. The second phase is a backward pass in which the error rates are propagated backward and the gradient descent technique is used to update the premise parameters.

3. Results and Discussion

Fig. 4 shows the absorption spectra in fluoxetine and sertraline. It indicates that there is a clear overlapping of the two spectra. This prevents the simultaneous determination of these drugs by direct visible absorbance measurements. To overcome this problem, multivariate techniques can be applied. Application of fuzzy ANFIS in multivariate calibration is proposed when significant non - linearities are observed in the data.

A suitable and simple technique for the non-linearity problem is the ANFIS method.

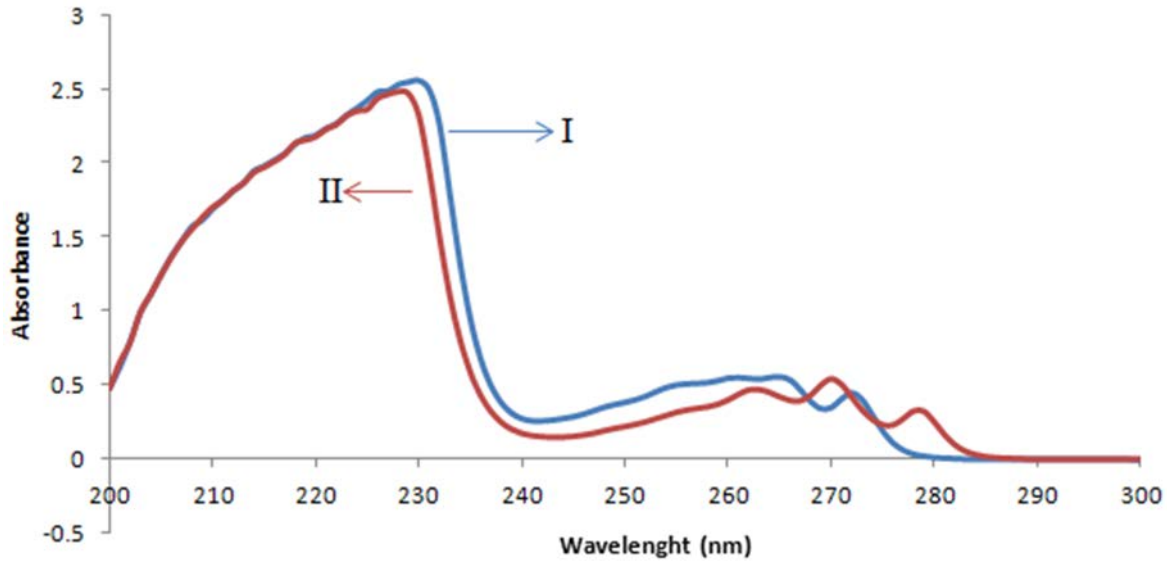


Figure 4. Absorbance Spectra of Fluoxetine 20µg. mL^{-1} (I) and Sertraline 20µg. mL^{-1}(II) in Ethanol.

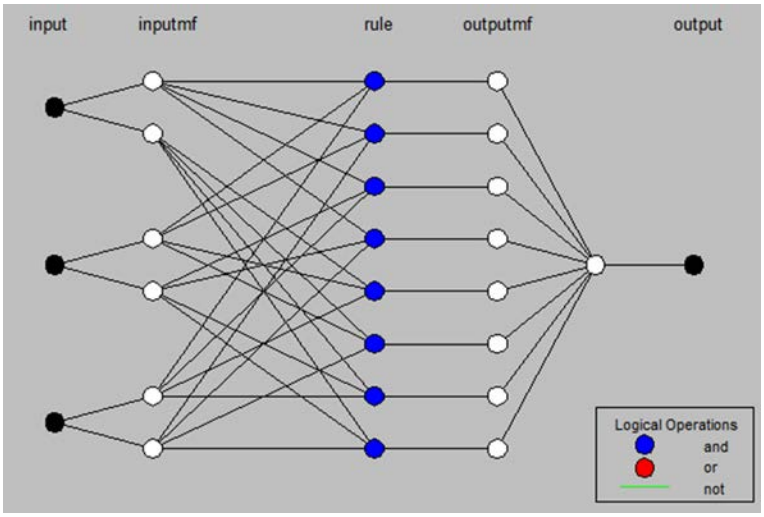


Figure 5. ANFIS Architecture is Show for a Three-input SugenoFuzzy Model With Eight Rules.

Spectra of mixtures of Fluoxetine and Sertraline solutions between 200 and 300 nm at 1nm intervals were recorded, and then the data were digitized and stored for data processing.

3.1. Effect of pH

Both FLX and SRT have amine groups. The effect of pH change was studied for these paration of spectral. The pH changes of the samples had no effect in separation or reduction of overlapping spectra. Due to their mutual interference, simultaneous determination of the binary mixture of FLX and SRT is not possible by using classical spectrophotometric method. In order to overcome this problem, we proposed the ANFIS and spectrophotometry methods.

3.2. ANFIS Model

In this step, the data set is divided into three subsets: the training, validation and test subsets. The training and validation sets were used for the construction of the ANFIS model, which validation set was used to prevent the overtraining/over fitting problem on training set and then the generated model was applied to the test set. Fig. 5 shows the architecture of the best ANFIS model, with two Gaussian MFs for inputs1–2 and one Gaussian MFs for input 3 that has the lowest testing error. The ANFIS was trained for 10 epochs.

The last part of the modeling process is to develop an ANFIS model using the concentrations appearing in the best model as inputs. ANFIS modeling involves two phases: structure identification and parameter identification. The former is related to find a suitable number of rules and a proper partition of the feature space.

The latter is concerned with the adjustment of system parameters, such as MF (membership function) parameters, linear coefficients, and so on. It is concluded that by increasing the number of MFs per input the number of rules increases accordingly.

The number and type of the membership functions needed for developing the ANFIS

Table 1. Statistical parameters of the optimized matrix using the ANFIS.

Parameters	FLX	SRT
	R^2	
Training set	0.9681	0.9726
Test set	0.9924	0.9944
Validation set	0.9837	0.9961
	RMSE	
Training set	1.9064	2.5345
Test set	1.4776	1.7926
Validation set	1.5092	2.4506

model were optimized based on the RMSE for the validation set (control set). The architecture for the optimized ANFIS model is shown in Fig. 3. The model was trained after 10 epochs. Finally, the optimized model was applied to all data sets and the statistical results obtained are shown in Table 1.As seen in "Table 1" R^2 of train is lower than R^2 of test, because the input traning value is greater than the value of the input test.

3.3. Determination of Fluoxetine and Sertraline in Synthetic Mixtures

The predictive ability of method was determined using thirty two component fluoxetine and sertraline mixtures (their compositions are given in Table 2). Because of the concentration selection the amount of SRT produced from the Dr. Abidi company is more than 4 times the amount of FLX.The results obtained by ANFIS to thirty synthetic samples are also listed in Table 2, as well as the recovery for the prediction series of fluoxetine and sertraline mixtures. As can be seen, the recovery was also quite acceptable. The plots of the predicted concentration versus actual values are shown in Fig. 6 for fluoxetine and sertraline, as well as the line equations and R^2 values.

3.4. Method Validation

The validation method was applied to the determination of FLX and SRT in commercially tablets. For this purpose, three

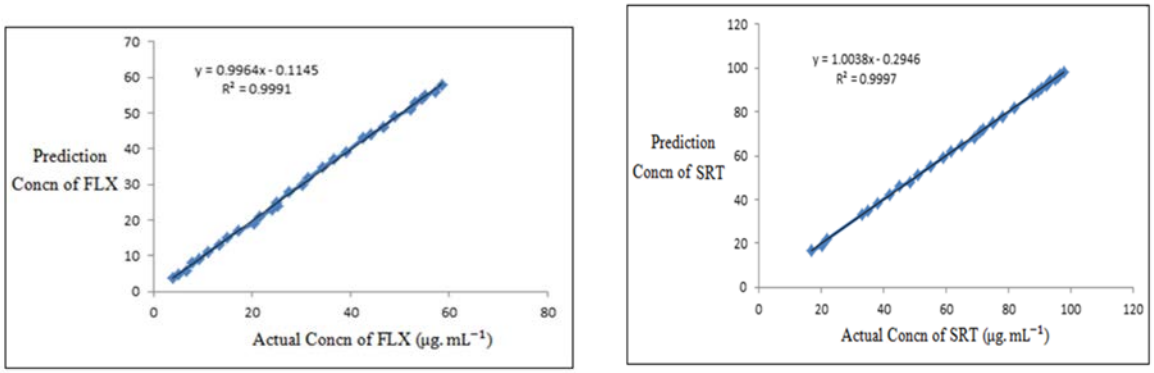


Figure 6. Plots of Predicted Concentration Versus Actual Concentration for Fluoxetine (FLX) andSertraline (SRT) by ANFIS (µg. mL^{-1}).

Table 2. Composition of synthetic samples (in $\mu g.ml^{-1}$) of fluoxetine and sertraline, and prediction by ANFIS model.

Sample	Fluoxetine ($\mu g.mL^{-1}$)		Sertraline ($\mu g.mL^{-1}$)	
	Actual	Found	Actual	Found
1	4	4.00	17	17.00
2	5	5.01	19	20.41
3	6	6.71	22	22.00
4	8	7.91	33	32.99
5	9	9.22	35	35.00
6	11	10.93	38	38.04
7	13	13.25	42	41.99
8	15	14.91	46	45.17
9	17	17.12	48	48.47
10	19	20.32	51	51.09
11	21	21.60	55	54.99
12	23	23.98	59	58.98
13	24	25.16	62	61.72
14	25	24.86	65	65.09
15	28	27.49	68	69.21
16	30	30.13	71	71.03
17	32	31.31	72	71.99
18	35	34.41	75	75.00
19	37	36.68	78	78.06
20	39	39.21	82	81.79
21	43	42.54	88	87.94
22	44	44.06	89	89.35
23	46	46.66	91	90.66
24	49	49.02	92	92.37
25	51	52.11	93	92.82
26	53	52.94	94	93.49
27	54	54.31	95	95.14
28	55	55.20	96	95.91
29	56	57.11	97	96.72
30	58	58.62	98	98.01
Mean Recovery	101.26		100.24	
RMSE	0.57		0.42	
RSD	0.46		0.38	
LOD	0.58		0.93	

spiked samples were prepared by adding aliquots (4$\mu g.\ mL^{-1}$) of FLX and SRT solution to the commercial formulation (Standard addition).

Mean recoveries and the relative errors of prediction were calculated and their results were given in Table 3. An estimation of the relative errors of prediction (REP %), using the following equation, for each component was made by cross-validation. The accuracy and reproducibility is evident from the data as mean recoveries are close to 100% and low root mean square error.

Eq 15

$$REP(\%) = \left[\frac{\sum_{i=1}^{n} (\overline{X_i} - X_i)^2}{\sum_{i=1}^{n} (X_i)^2} \right]^{1/2} \times 100$$

Table 3. Recovery result obtained from the standard addition technique by the application of the proposed method.

Sample	Fluoxetine ($\mu g.mL^{-1}$)			Sertraline ($\mu g.mL^{-1}$)		
	Add	Found	REP (%)	Add	Found	REP (%)
1	4	4.01	0.25	4	3.74	6.50
2	4	3.90	2.50	4	4.16	4.00
3	4	3.84	4.00	4	4.11	2.75
Mean Recovery		109.33			115.44	

Table 4. Assayed result of simultaneous determination of FLX and SRT in tablets by the ANFIS model.

Sample	FLX ($\mu g.mL^{-1}$)	SRT ($\mu g.mL^{-1}$)
1	20.53	100.09
2	20.21	100.96
3	20.39	99.45
Amount on the label (mg)	20	100
Mean Recovery	101.88	101.16
RMSE	0.20	0.28
S. D[a]	0.131	0.618
RSD (%) [b]	0.643	0.617

[a]Standard Deviation.

[b]Relative Standard Deviation.

3.5. Result for Real Sample

In order to assess the applicability of the proposed method to the analysis of real samples, it was applied to the determination of these drugs in pharmaceutical formulation. Three replicate measurements were made. Absorption spectra of commercial dosage of fluoxetine and sertraline were prepared similar to the details described in the experimental section. Data extracted from the absorption spectra then were fed into the established network. The output of the ANFIS is summarized in Table 4. The reasonable RSE (%) for each analyte indicates that the accuracy of the proposed method is reasonable; also the good agreement between these results and label claim indicates the successful applicability of Fluoxetine and Sertraline in real sample. Moreover, comparing the spectra obtaining from the mixture of FLX and SRT in standard and drug formulation solutions shows similar patterns in their spectra (Fig. 7). These findings indicate that the excipients placed in commercial preparation did not significantly interfere in the measurement of FLX and SRT in pharmaceutical formulation.

3.6. Determination of Fluoxetine and Sertraline in Pharmaceutical Formulations and Biological Fluids

In order to show the analytical applicability of the proposed methods, first calibration curve obtained from adaptive neuro-fuzzy inference system (ANFIS) model at PH 2 were applied to determination of fluoxetine

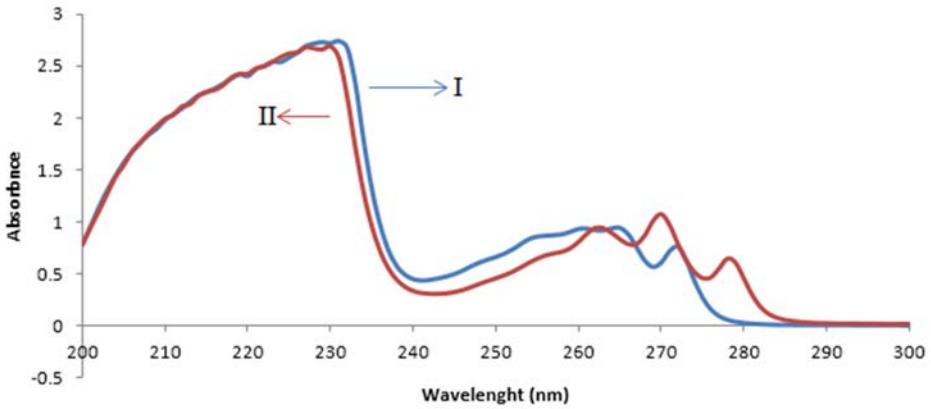


Figure 7. Absorption Spectra of Fluoxetine 20µg. mL^{-1}(I) and Sertraline 20µg. mL^{-1}(II) Tablets in Ethanol.

and sertraline in real samples (pharmaceutical formulations) and complex matrices, i.e. urine . The results showed that satisfactory recovery for fluoxetine and sertraline could be obtained (Tables 5) using the recommended procedures. To determine the effectiveness of a method (and also of the working range), recovery experiments can be carried out. Recovery can be defined as the 'fraction of the analyte determined after addition of a known amount of the analyte to a sample'. In practice, control samples are most commonly used for spiking.

The recovery is calculated with:

Eq 16 Recovery=$\frac{A}{B} \times 100$Where

A= The amount obtained B= The actual amount

Results of the determination are summarized in Table 5. The data obtained by these methods reveal the capability of the methods for determination of fluoxetine and sertraline in real samples such as pharmaceutical formulations and complex matrices such as urine without considerable error. The average recoveries in pharmaceutical formulations (Drabidi Tablets) and complex matrice (urine) are summarized in Table 5.

3.7. Analysis of Commercial Formulation

The results achieved by the proposed method were compared with each other using one-way ANOVA test. The value of F-critical for comparison with the F-calculated is used. The F-calculated values (P = 0.05) were less than the tabulated F-values. No significant differences were observed between the results of the proposed method at 95% confidence level. The corresponding results are summarized in Table 6.

Findings of our study suggest that, there are no significant errors in simultaneous

Table 5. Determination of fluoxetine and sertraline in urine using ANFISmodel .

	Added (µg. mL^{-1})	Found (µg. mL^{-1})	Recovery (%)
Urine sample 1	1.50	1.42	94.7
Urine sample 2	3	3.31	94.6

Table 6. The ANOVA results by applying the proposed methods to the real sample.

Source of Variation	Sum of squares (SS)	Degree of freedom	Mean squares (MS)	F	P-value	F crit
Between Groups						
Fluoxetine	0.09	1	0.09	11.11111	0.079425	18.51282
Sertraline	0.042025	1	0.042025	0.073725	0.811448	18.51282
Within Groups						
Fluoxetine	0.0162	2	0.018			
Sertraline	1.14005	2	0.570025			
Total						
Fluoxetine	0.1062	3				
Sertraline	1.182075	3				

determination of FLX and SRT by proposed methods.

4. Conclusion

Since the absorption spectra of binary mixture of fluoxetine and sertraline strongly overlap with each other, we used the ANFIS methods combined with UV-Vis spectrophotometer technique.

Determination of fluoxetine and sertraline with the ANFIS method was established, with good prediction ability in the synthetic mixtures. Results show that ANFIS is a suitable calibration method for the simultaneous spectrophotometric determination of fluoxetine and sertraline.

These are rapid procedures which only require the solution of the sample and followed by measurement of its UV-Vis spectrum. So they are simple, inexpensive and very fast procedures which neither need a previous separation of the analytes nor other previous sample treatments. Although other methods such as chromatographic methods can be used to determine these components in pharmaceuticals, they are both more time consuming and expensive than the procedure here developed.

The best recovery values are obtained by application of the ANFIS model to absorbance data. The good agreement clearly demonstrates the usefulness of this procedure for the simultaneous determination of fluoxetine and sertraline in synthetic and real samples.

Acknowledgement

The authors would like to acknowledge the Faculty of Chemistry, Azad University, North Tehran Branch for their support and contribution to this study.

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