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Resolving Spectra Overlapping Based on Net Analyte Signal for Simultaneous Spectrophotometric Determination of Fluoxetine and Sertraline

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The net analyte signal standard addition method was used for simultaneous spectrophotometric determination of sertraline and fluoxetine in pharmaceutical preparations. The method combines the advantages of the standard addition method with the net analyte signal concept to enable the extraction of information about an analyte from the spectra of multi-component mixtures. This method uses full spectrum realization and does not require calibration and prediction steps. Determination requires only a few measurements. The limit of detection for fluoxetine was 0.31 µg ml^{-1} and for sertraline was 0.20 µg ml^{-1}. The root mean square error for fluoxetine was 0.45 and for sertraline was 0.39.

Keywords: Fluoxetine, Sertraline, Antidepressant, Net analyte signal, Spectrophotometric

INTRODUCTION

Multivariate spectral calibrations are standard methods for quantitative spectral analysis. They allow the simultaneous determination of several analytes in a mixture [1]. The partial least squares and principle component regression methods are the most common multivariate calibration methods for quantitative spectral analysis [2-4]. These full spectrum multivariate calibration methods quickly determine the components of interest in mixtures and do not require separation steps for analysis.

A new family of multivariate calibration methods based on the concept of a net analyte signal (NAS) has been proposed [5-18]. NAS calibration for a multivariate problem was originally described by Lorber [19]. Based on Lorber's definition, the NAS for any analyte based on spectroscopic methods is the part of the spectrum that is orthogonal to the space spanned by the spectra of all constituents except the analyte (all interfering constituents) (Fig. 1). Since the NAS vector specifies only the direction affected by changes in analyte concentration, it can selectively determine an analyte. The method allows calculation of the analytical figures of merit and method performance, including limit of detection, sensitivity and selectivity for each component [19].

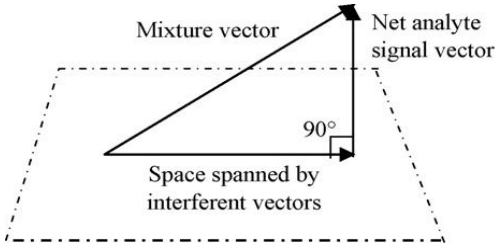

Fig. 1. Geometrical representation of the NAS vector. The NAS vector is orthogonal to the space spanned by the interferent vectors.

In a simple case, the norm of the NAS vector can be used to construct a univariate calibration model where this parameter is plotted against the analyte concentration and a linear relationship is observed. The application of the NAS to multicomponent spectroscopic analysis usually requires

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selection of spectral variables to build well-fitted models and avoid non-modeled interferences [20-22].

Training the multivariate calibration methods with selected spectral regions rather than a full-spectrum region allows the informative part of the spectrum related to the variation in concentration of the analyte to be modeled. The parts of the spectrum that are related to the variation in concentration of other analytes and background variations are discarded, which increases the performance of multivariate calibration model. Several approaches have been proposed for selection of an optimal set of spectral regions for multivariate calibration, such as generalized simulating annealing [23], genetic algorithms [24], artificial noise introduction in PLS modeling [25], wavelet transforms [26], successive projections algorithms [27], and the moving windows selection strategy [28].

Depression is a chronic or recurrent mood disorder that affects the social and economic functioning of people the world over [29]. Fluoxetine HCl (FLX) is a selective serotonin reuptake inhibitor (SSRI) commonly prescribed to treat depression. It is chemically designated as N-methyl-3-phenyl-3-[4-(trifluoromethyl) phenoxy]propan-1-amine (Fig. 2a). As a SSRI, it acts by increasing the extracellular level of the neurotransmitter serotonin by inhibiting its reuptake into the cells [30]. Fluoxetine is used to treatment major depression (including pediatric depression), panic disorders and premenstrual dysphonic disorder. It has also been used for cataplexy, obesity and alcohol dependence [31].

Sertraline [(1S,4S)N-methyl-4-(3,4-dichloro phenyl)-1,2,3,4-tetrahydro-1-naphthylamine, SER] (Fig. 2b) is a second generation antidepressant, and also a SSRI. It has been approved by the USFDA for the treatment of depression, obsessive-compulsive disorder, posttraumatic stress disorder, social anxiety disorder, postmenopausal dysphoric disorder, and panic disorder [32].

Several methods have been proposed to determine the presence of fluoxetine (I) in biological fluids and pharmaceutical formulations, including HPLC [33-40] and GC [40-44]. The presence of sertraline hydrochloride (II) has been determined in biological fluids and dosage forms using GC mass [44-47], GC [48], and HPLC [49-52].

The present study describes a sensitive, selective, accurate and inexpensive procedure for simultaneous spectrophotometric determination of the presence of fluoxetine and sertraline using the net analyte signal standard addition method (NASSAM). This is a standard addition method that uses the NAS to calculate the NAS vectors and attribute them to a specific concentration of analyte using UV-Vis spectrophotometry.

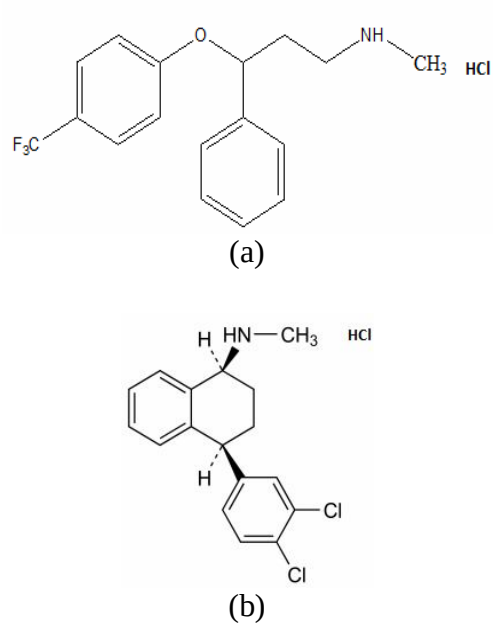


Fig. 2. The structures of fluoxetine HCl (a) and sertraline HCl (b).

THEORY

Figure 3 shows the development of the control charts where a sample spectrum (vector r) is split into three contributions: r_{NAS} (NAS vector), r_{INT} (interference vector), and r_{res} (residual vector). The NAS vector corresponds to the analyte of interest and all the non-relevant information of the analyte is explained by the interference vector. Information that cannot be explained by the NAS or the interference vectors are considered to be in the residual vector. The subspace is called the interference space. Spectra of blank samples or pure interferences can be used to span the interference space.

Conventional notation has been used throughout. Boldface capital letters denote the matrix, boldface lowercase letters denote the column vectors, and lightface

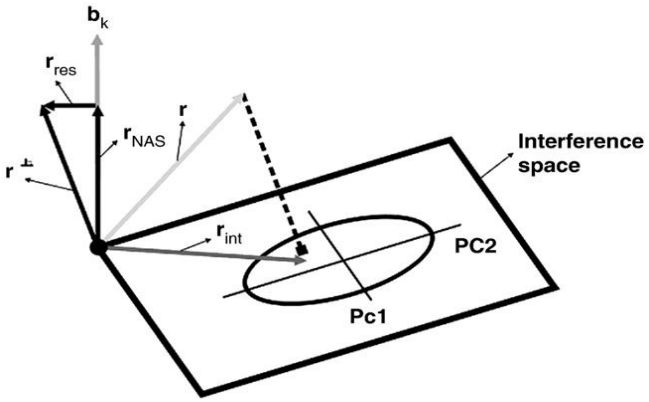


Fig. 3. Schematic overview of the separation of the spectrum r into three different contributions, the NAS values indicated by the vector r_{NAS}, the composition of the excipients indicated by the vector r_{INT} in the interference space, and the residuals indicated by vector r_{res}, perpendicular to of the regression vector NAS b_k.

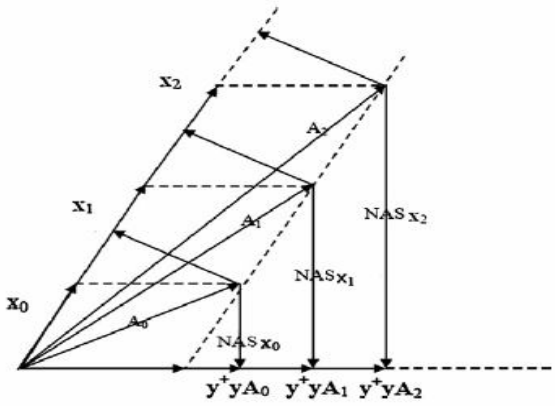


Fig. 4. Representation in two dimensional vector space analyte (**x**) and vector of interfering agent (**y**) and NAS vector NAS**x** will be different from x in direction and length. The vector $y^+ y\boldsymbol{A_i}$ is the part of A in the interference space.

lower case italic letters denote scalar values. Superscript T denotes the operation of a vector or matrix transposition and superscript + denotes the pseudo-inverse of a non-square matrix. A digitized spectrum is referred to as a spectrum vector or simply as a vector; the spectrum vector of a pure component is called a component vector.

Consider an unknown sample containing analyte X and interferent Y. Determination of the concentration of X in a sample solution using NASSAM in the presence of Y requires the spectrum vector of Y. Known amounts of X are successively added to the sample solution and the resulting absorbance is measured and expressed as:

$$A_0 = e_x c_x + e_y c_y \quad (1)$$

$$A_1 = A_0 + e_x c_{xsI} \quad (2)$$

$$A_i = A_{i-1} + e_x c_{xsi} \quad (3)$$

$$A_n = A_{n-1} + e_x c_{xsn} \quad (4)$$

where A_0 and A_i are the analytical signals for the unknown sample before and after addition of the known amounts of X, respectively. The NAS of the X component, $NASX_i$, can be found using the following orthogonal projection:

$$NASx_i = (I - y^+ y)A_i \quad (5)$$

By definition, it is always possible to split the spectrum of a sample (**A**i) into two distinct parts: $NASX_i$, which is orthogonal to the spectra of the interferences and $y^+ y\boldsymbol{A_i}$, the part of the spectrum that can be generated by linear combination of the spectra of the interfering agents. Consequently, $y^+ y\boldsymbol{A_i}$ cannot be unique for the analyte of interest because it can be also produced by a mixture of interfering agents. $NASX_i$ is orthogonal to the spectra of the interferences and reflects the part of the spectrum that is dependent only on analyte X in the mixture. This part, called the net analyte signal vector ($NASx_i$), can be used to quantify analyte X [53-55].

Figure 4 shows the geometrical presentation of the analyte, interferent, mixtures, $y^+ y\boldsymbol{A_i}$ and $NASx_i$ vectors. The shape of $NASx_i$ depends solely on the presence of interferences in the mixture, not on their specific concentrations. Only the addition or deletion of the components can alter this value. It is assumed that the spectra of samples without analyte are available and remain constant during determination. In binary and/or ternary

mixtures, when the interferences are known, the NAS can be calculated for each analyte. The norm of the NAS vector can be used to construct a univariate calibration model. This parameter can be plotted against the analyte concentration results in a linear relationship. If a matrix effect exists, standard addition plots can be constructed. The standard addition method based on NAS is used here to eliminate the calibration and prediction steps of multivariate calibration methods. Determination is carried out in a single step for each analyte.

EXPERIMENTAL

Reagent and Chemicals

Ethanol (analytical UV-grade) was purchased from Merck (Germany). Pure fluoxetine (FLX) and sertraline (SRT) and their pharmaceutical dosage forms containing 20 mg of FLX and 100 mg of SRT were donated by Dr. Abidi Pharmacy (Tehran, Iran). Sulfuric acid and NaOH were purchased from Merck (Germany).

Instruments and software. Spectrophotometric analysis was carried out using a Shimadzu UV-2100 UV-Vis double beam spectrophotometer equipped with 1.0 cm quartz cells. The absorption spectra of the solutions were recorded at 200 to 300 nm at 1 nm intervals with respect to a blank of ethanol. The spectrophotometric measurements were carried out at room temperature (20 °C) and all solutions were prepared fresh on the day of analysis.

A pH meter (model pHs-3C) was calibrated using buffer solution and then used for pH measurement. The pH of the solution was surveyed at pH = 2.0-9.0. The data was processed using the NAS of MATLAB 7.12 and Microsoft Office Excel 2010 on a Pentium IV personal computer.

Preparation of the standard solution. Standard stock solution was prepared by dissolving 20 mg of each component in ethanol and then adding the same solvent to dilute the mixture to 100 ml in a volumetric flask. Standard solutions were prepared by appropriate dilution of stock solution at 5-120 μg ml^{-1} for FLX and 10-120 μg ml^{-1} SRT for the calibration set.

Preparation of the real sample. In order to assay the pharmaceutical forms, 10 tablets were weighed individually to obtain their representative average weights. The tablets were then finely powdered and mixed. A mass corresponding to one tablet was accurately weighed and transferred to a 250 ml^{-1} beaker and dissolved in 100 ml^{-1} ethanol. The solution was stirred using a magnetic stirrer for 30 min and filtered through a Whattman no. 41 filter paper. After filtration, the clear solution was adjusted to a volume of 100 ml using the same solvent. This solution was further diluted to suitable concentrations for UV measurement [56].

RESULTS AND DISCUSSION

UV Spectra and Proposed Methods

Figure 5 shows the absorption spectra of FLX and SRT under experimental conditions. As shown, the maximum wavelengths of the two compounds are similar and their spectra overlap at 200 to 300 nm. The effect of pH was studied for the separation of spectra. Changes in the pH of the samples had no effect on the separation or decrease of the area of overlap of spectra. The mutual interference of FLX and SRT meant that simultaneous determination of the binary mixture was not possible using classical spectrophotometry.

NASSAM was the chemometric method used to simultaneously resolve the matrix effect and interferent errors. Standard solutions of the two drugs were prepared and maximum information for each compound was obtained using a few samples of each. Table 1 lists the concentrations of the standard solutions.

Construction of the Calibration Graph

The wavelength of the spectra can affect the accuracy of the proposed method. When the range of wavelengths was wide, excess noise on the spectra was at times included in the modeling. At a narrow range of wavelengths, the spectra of the components were at times lost.

Under optimum conditions, the norm of the NAS vectors (Y) increased linearly by the concentrations (C) of 5.0 to 120.0 μg ml^{-1}using a calibration equations of Y = 0.0041 C + 0.0917 (R^2 = 0.9825) for FLX and Y = 0.0012 C + 0.02583 (R^2 = 0.9843) for SRT. The limit of detection was 3 S_b, where S_b is the standard deviation of several replicated measurements of analyte at zero concentration. The values obtained for FLX and SRT were 0.31 and 0.20 μg ml^{-1}, respectively. The mean recovery (%) for determination of FLX was 99.19% and for SRT was 100.24%.

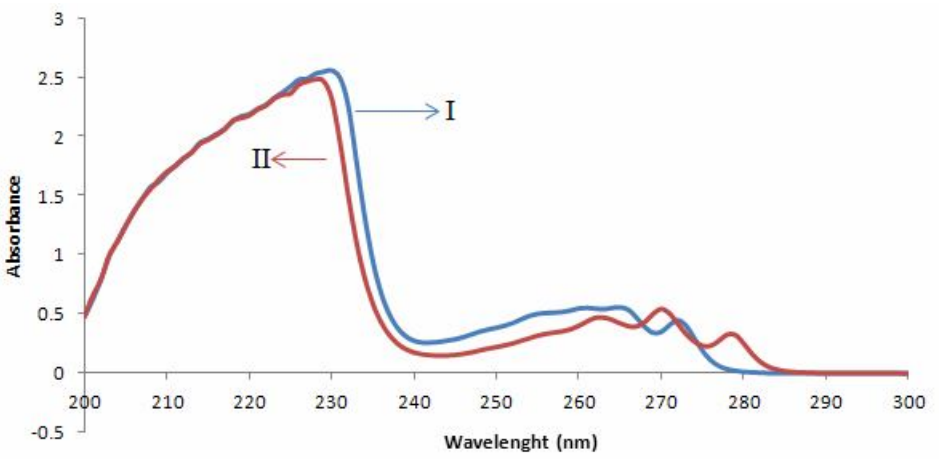


Fig. 5. Absorbance spectra of fluoxetine 20μ g ml^{-1} (I) and sertraline 20μ g ml^{-1} (II) in ethanol.

Table 1. Composition of the Calibration Sample

Sample	Fluoxetine (ml)	Sertraline (ml)
1	5	10
2	10	15
3	20	10
4	50	50
5	100	100
6	120	120

NAS Modeling

Multivariate calibration methods employing the concept of net analyte signal (NAS) have been introduced, which may deserve some comments. They attempt to define, from a multicomponent spectrum R, a net analyte vector R* which is uniquely related to the concentration of the analyte of interest k. In mathematical terms, the NAS for analyte k (R*) is defined as the part of a spectrum r which is orthogonal to the space spanned by the spectra of all other analytes in the sample. For inverse calibration methods, it is given by the following orthogonal projection:

$$R^* = [\mathrm{I} - (R_{-K})^{+} R_{-K}]\mathrm{R} \qquad (6)$$

where I is a $J \times J$ unitary matrix, R_{-K} is an $I \times J$ matrix representing the space spanned by the spectra of all other analytes except k, R_{-K}^{+} is its pseudoinverse and $P_{NAS,K}$ is a $J \times J$ matrix projecting a given vector on to the NAS space . The pseudoinverse R_{-K}^{+} is computed by singular value decomposition of R_{-K} using A spectral factors, with A usually estimated by cross-validation techniques (see below). Eq. (1) thus becomes:

$$R^* = (\mathrm{I} - \mathrm{P}\,\mathrm{P}^{T})\,\mathrm{R} \qquad (7)$$

where P is a $J \times A$ matrix at which columns are the first significant A eigenvectors of the square matrix $[([R_{-K})]^{T} R_{-K}]$, *i.e.*, those associated with the largest A eigenvalues.

To determine the applicability of NASSAM for spectrophotometric analysis, binary mixtures of FLX and

SRT were prepared and standard solutions of each were added to individual mixtures. Figures 6a for FLX and 6b for SRT show the Rstar curves. NAS was applied to the raw spectra to extract more subtle information related to the variation in FLX and SRT concentrations.

To demonstrate how the NAS correlation spectra indicate more distinctive features than the conventional correlation spectra, we systematically varied the principal factor parameter f, as f = 1, 2 and 3. With increasing f, more information contributed by the analyte of interest is extracted. Ideally no other information is included to the reconstructed spectra but in the real situation there exists an optimal principal factor.

The spectra reconstructed with the optimal principal factor include sufficient information from the analyte of interest whereas minimizing unrelated part of the spectra.

We applied NAS to the raw spectra, to extract more subtle information directly related to the Fluoxetine and Sertraline Hydrochloride concentration variations. Figure 7 shows principal factor *f* at *f* = 1, 2 and 3. The first three principal factors represent 99.5% of the variation of the original spectra and remaining variation was considered to be interference and noise. The reconstructed spectra are shown in Figs. 7a to 7c. Table 2 shows the utility of the method as evaluated by the determination of FLX and SRT content in samples at different concentration ratios.

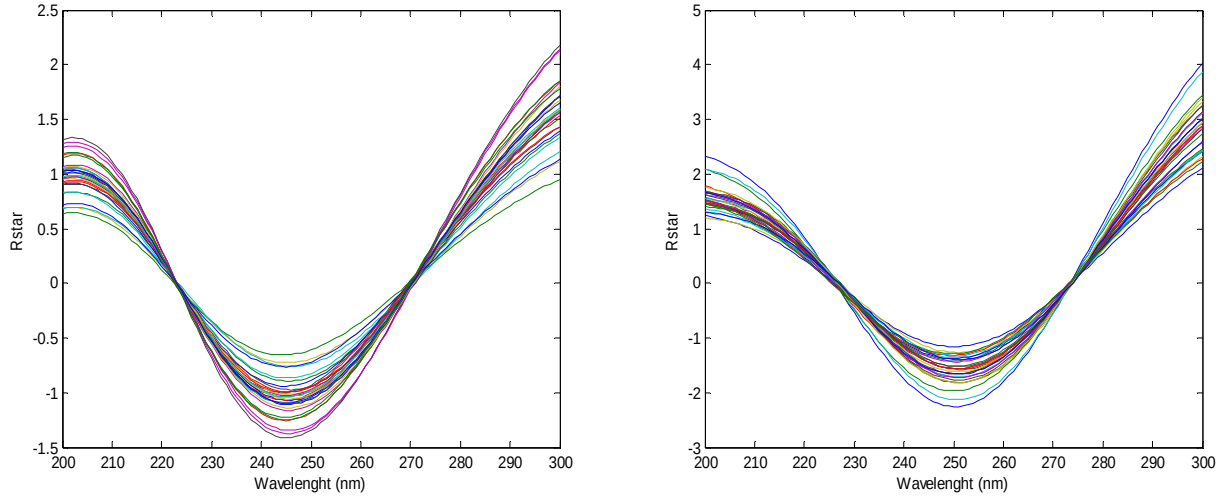
Method Validation

The method was validated by determining the FLX and SRT content of tablets. Three spiked samples were prepared by adding aliquots of 2 μg ml^{-1} FLX and SRT solution to the commercial formulation (standard addition) [38,42]. The recoveries for each drug and mean recovery were calculated using the concentration obtained from the spiked mixtures and those from the pure drug. Table 3 shows that the efficacy of the procedure was satisfactory.

Analysis of the Commercial Tablets

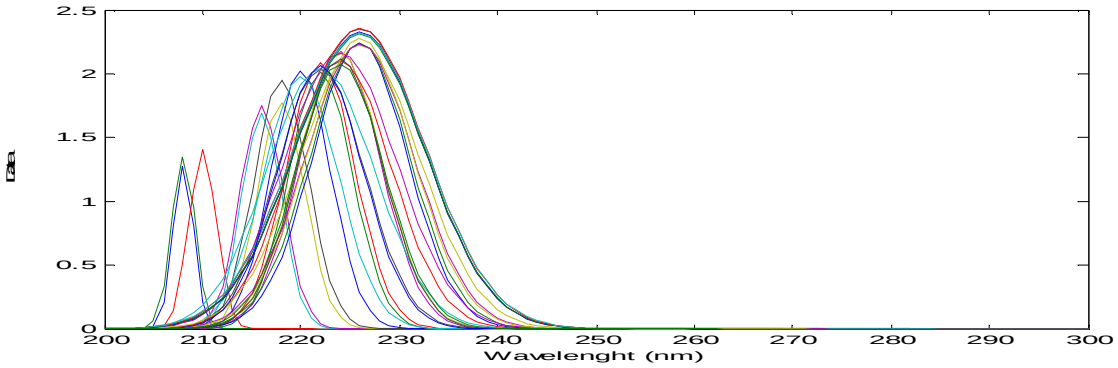
The performance of the proposed method was evaluated by assaying commercial FLX and SRT tablets. Table 4 shows the results of application of NASSAM to the pharmaceutical formulations of FLX and SRT. The reasonable RSE (%) values for each analyte confirm the accuracy of the proposed method. There was good agreement between the results and the dosages on the manufacturer labels, indicating the accurate determination of FLX and SRT in real samples. Moreover, a comparison of the spectra from the FLX and SRT in standard and drug formulation solutions shows similar spectral patterns (Fig. 8).

The RSE for prediction of the concentration of a single component in a mixture was calculated as:

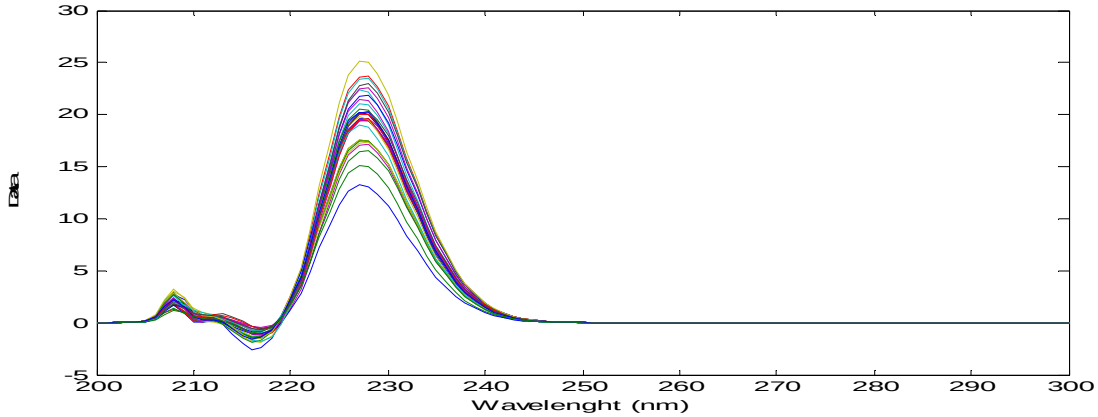


(a) (b)

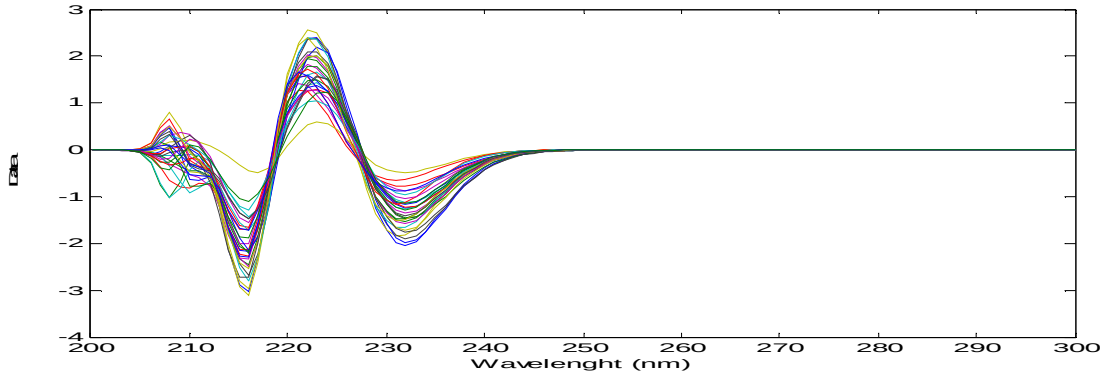
Fig. 6. Rstar plot for (A) FLX and (B) SRT.



F = 1



F = 2



F = 3

Fig. 7. Reconstructed spectra manipulated with the NAS with principle factor: (a) f = 1; (b) f = 2; (c) f = 3.

Table 2. Simultaneous dDtermination of FLX and SRT in some Synthetic Mixtures

Sample	Fluoxetine		Sertraline	
	Found	Actual	Found	Actual
1	4	3.79	17	16.87
2	5	5.00	19	19.38
3	6	5.90	22	22.00
4	8	7.72	33	33.56
5	9	8.53	35	34.62
6	11	10.41	38	38.27
7	13	13.17	42	42.44
8	15	14.63	46	45.89
9	17	17.00	48	47.92
10	19	19.30	51	51.00
11	21	21.26	55	55.73
12	23	22.68	59	59.38
13	24	23.44	62	61.14
14	25	25.10	65	65.00
15	28	28.00	68	68.49
16	30	30.33	71	71.18
17	32	31.23	72	72.53
18	35	33.87	75	75.36
19	37	37.00	78	78.72
20	39	38.80	82	81.93
21	43	43.41	88	88.70
22	44	43.84	89	89.01
23	46	45.53	91	91.20
24	49	48.79	92	91.80
25	51	51.42	93	92.31
26	53	53.27	94	94.09
27	54	54.61	95	95.26
28	55	54.94	96	95.92
29	56	57.28	97	97.14
30	58	58.01	98	98.11
Mean Recovery	99.19		100.24	
RMSE	0.45		0.39	
LOD	0.31		0.20	
RSD (%)	0.57		0.38	

Table 3. Recovery Result Obtained from the Standard Addition Technique by the Application of the Proposed Method

Spiked sample	Added (μg ml^{-1})		Found (μg ml^{-1})		Recovery (%)	
	FLX	SRT	FLX	SRT	FLX	SRT
1	2.00	2.00	2.20	2.30	110.00	115.00
2	2.00	2.00	1.92	1.95	96.00	97.50
3	2.00	2.00	2.18	2.18	109.00	109.00
Mean recovery (%)					105.00	107.17

Table 4. Assayed Result of Simultaneous Determination of FLX and SRT in Commercial Tablet Using the NAS Model

Sample	Fluoxetine					Sertraline				
	Actual	Found	RSE (%)	MR[a]	RSD (%)[b]	Actual	Found	RSE (%)	MR	RSD (%)
1	20	19.51	2.45	99.00	0.030	100	98.61	1.39	99.70	0.007
2	20	19.26	3.70			100	99.15	0.85		
3	20	20.63	3.15			100	100.34	1.34		

[a]Mean Recovery. [b]Relative Standard Deviation.

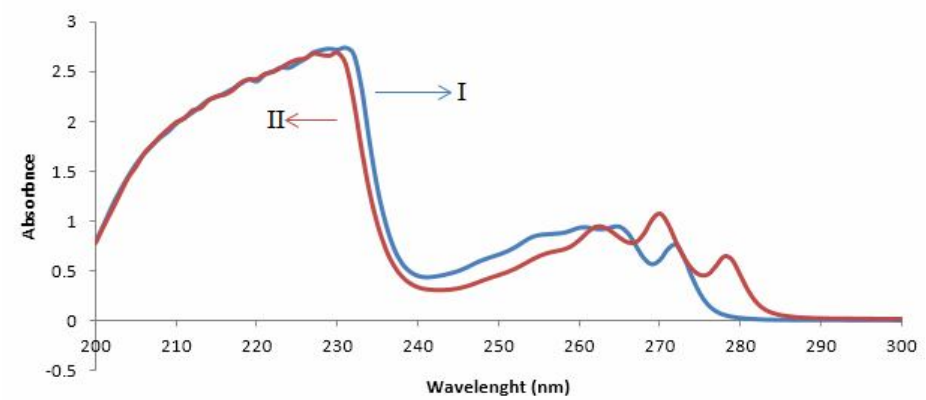


Fig. 8. Absorption spectra of fluoxetine 20 µg ml^{-1} (I) and sertraline 20 µg ml^{-1} (II) tablets in ethanol.

$$R.S.E(\%) = \left(\frac{\sum_{j=1}^{N} (C_j^A - C_j)^2}{\sum_{j=1}^{N} (C_j)^2} \right)^{1/2} \times 100 \qquad (8)$$

where N is the number of samples; C_i is the concentration of the component in the j^{th} mixture; and C_j^A is the predicted concentration.

ANOVA

The proposed method was applied to simultaneous quantitative determination of FLX and SRT in tablets and the results are listed in Table 5. One-way ANOVA was applied to determine the existence of a significant difference between the experimental results and label dosage.

The calculated F-values were lower than the critical F-values, thus, it was concluded that there were no significant errors (95% confidence level) in determination of the amount of FLX and SRT. This indicates that the proposed method was sufficiently accurate for application to pharmaceutical dosages. Table 6 compares predicted concentrations of NAS and experimental concentrations of FLX and SRT. It indicates good correlation between the real values and those predicted by NAS.

CONCLUSIONS

Chemometric calibration techniques are commonly used for spectral analysis and quality control of drugs in mixtures and multicomponent formulations with overlapping spectra, because separation of the components is not required. This study confirmed the potential usefulness of NAS methods combined with UV-Vis spectrophotometry for simultaneous quantitative analysis of FLX and SRT in pharmaceutical formulations.

Prediction of the compound concentrations was facilitated by the use of an orthogonal design to build a calibration data set that was then used to calibrate the NAS model. The proposed procedure simultaneously quantified

Table 5. The ANOVA Results by Applying the Proposed Methods to the Real Sample

Source of Variation	SS	df	MS	F	P-value	F crit
			Between Groups			
Fluoxetine	0.003025	1	0.003025	0.006447	0.943316	18.51282
Sertraline	0.060025	1	0.060025	0.050062	0.843733	18.51282
			Within Groups			
Fluoxetine	0.93845	2	0.469225			
Sertraline	2.39805	2	1.199025			
			Total			
Fluoxetine	0.941475	3				
Sertraline	2.458075	3				

ss, sum of squares; df, degree of freedom; MS, mean squares.

Table 6. Statistical Results of Actual and Predicted Concentrations of Fluoxetine and Sertraline by Net Analyte Signal

Parameter	FLX	SRT
Slope[a]	1.0092	0.9994
Intercept[b]	-0.3369	0.1679
Regression coefficient (R^2)	0.9994	0.9998
Correlation coefficient (r)	0.9996	0.9998

[a]Slope of the regression function. [b]Intercept of the regression function; R^2, Regression coefficient; r, Correlation coefficient of the regression function.

both analytes with acceptable error for concentration level. The analytical performance of the chemometric method was characterized by RSE (%) for prediction of concentration. The proposed method was shown to be simple, fast, and inexpensive and does not require initial pretreatment steps, making it appropriate for quality control laboratories.

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