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Stability Indicating Spectrophotometric and Chemometric Methods for Determination of Nifuroxazide in Presence of Its Alkaline Degradation Products

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Abstract

Simple, accurate, sensitive and precise Spectrophotometric and Chemometric methods were developed and validated for determination of Nifuroxazide (Nx) in presence of its alkaline degradation products. Two spectrophotometric methods were investigated, the first one is a second derivative spectrophotometric (^{2}D) determination of Nx in presence of its alkaline degradation products by measuring the peak amplitude at λ=278 nm which corresponds to (zero crossing) of its degradates. While, the second one is a second derivative ratio spectra (^{2}DD) which could successfully determine without any interference from its degradates at λ=290.8, The percentage recoveries of pure was found to be 99.97±1.505 & 99.83±1.401 for (^{2}D) & (^{2}DD) respectively. Chemometric-assisted spectrophotometric methods were also applied as classical least squares (CLS), principal component regression (PCR) and partial least squares (PLS), the percentage recoveries for Nx were 99.49 ±2.60 , 98.914 ±1.68 and 99.08 ±1.38 for CLS, PCR and PLS, respectively. The developed chemometric models have the advantage that they are not only determine the drug in presence of its degradates but also quantify the degradates in the mixtures. All the developed methods were successfully applied for the determination of Nx in its bulk powder and dosage form and the validity of the proposed methods was further assessed by applying the standard addition technique. The developed methods were statistically compared with each other and with a reported method and no significant difference were observed regarding both accuracy and precision, all the developed methods have been validated according to ICH guidelines.

Keywords: Nifuroxazide; Stability indicating methods; Ratio derivative; Chemometrics; Spectrophotometry; Alkaline degradation

Introduction

Nifuroxazide (Nx) is a broad spectrum intestinal anti-infectious agent with a strictly local action suitable for diarrhea. It is chemically designated as 4-Hydroxybenzoic acid[(5-nitro-2-(furanyl)methylene] hydrazide [1], Figure 1. It is a synthetic derivative of the nitro furans group. It has the advantage of rapid action, stopping diarrhea of infectious origin within 24-48 hours at most, and is active against the majority of organisms known to provoke diarrhea [2]. It's liable to alkaline hydrolysis using 0.1N NaOH under heating for 2hrs at 70°C to give the assumed degradation products, Figure 2 [3]. Different methods have been reported for the determination of Nx in biological fluids either in pharmaceutical dosage forms, in combination with other drugs or in the presence of its metabolites; these methods include spectrophotometry [4,5] liquid chromatography-UV photodiode array detection [6], high performance liquid chromatography (HPLC) [7-9], voltammetry [10-13] differential pulse polarography [14] and colorimetry [7]. The scientific novelty of the present work is that the methods used are simple, rapid, selective, less expensive and less time consuming compared with other published LC, TLC and HPLC methods, furthermore these methods could determine the intact drug without any interference from its degradation products. So, the aim of this work was to develop simple, sensitive and validated spectrophotometric and chemometric methods for the determination of Nx in the presence of its degradation product in powdered forms, laboratory prepared mixtures and in pharmaceutical formulation. The applied methods are second derivative (2D) and second derivative ratio (2DD), and chemometric methods as classical least squares (CLS), principal component regression (PCR) and partial least squares (PLS).

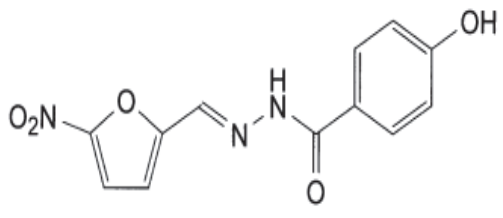

Figure 1: Nifuroxazide.

Experimental

Instruments

A double-beam uv–visible spectrophotometer (shimadzu, japan) model uv-1650 pc with quartz cell of 1 cm path length, connected to an ibm-compatible computer.

The software was uv-prob personal spectroscopy software version 2.21.

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The spectral bandwidth was 2 nm and wavelength-scanning speed 2800 nm/min. A uv lamp with a short wavelength(254 nm).

Materials

Pure standard: Nifuroxazide was kindly supplied by El Hekma Pharmaceuticals, 6th of October City, Cairo, A. R. E.

Recovery percentage was 99.63% according to manufacturer method of assay.

Pharmaceutical dosage forms: Antinal® capsules, batch no. 2488, were labeled as containing 200 mg of Nx manufactured by Amon Pharmaceuticals, El-Obour City, Cairo, A.R.E. and were obtained from the local market.

Chemicals and reagents: The ethanol used was of spectroscopic grade and was purchased from Prolabo (VWR International, West Chester, Pennsylvania). Sodium Hydroxide (El-Nasr pharmaceutical chemicals co.) 0.1N aqueous solution and Hydrochloric acid (El-Nasr pharmaceutical chemicals co.) 0.1N aqueous solution were used in the study.

Standard solutions

Stock standard solution of Nx (0.2 mg mL^{-1}) was prepared by accurately weighing 20 mg of Nx powder in 100 mL ethanol .

Stock Solution of Nx degraded (equivalent to 0.2 mg mL^{-1} Nx) was prepared by dissolving 20 mg of Nx in 30 ml 0.1N NaOH and heating in an oven for 2hr at 70°C then cooling, the solution was then neutralized with 0.1N HCl, the volume was completed to 100 mL with ethanol.

Working standard solution of Nx (0.02 mg mL^{-1}) was prepared by an additional dilution of its stock standard solution with ethanol for the spectrophotometric methods.

Working solution of Nx (0.1 mg mL^{-1}) was prepared by an additional dilution of its stock solutions with ethanol for the chemometric methods.

Working solution of Nx degraded (0.02 mg mL^{-1}) was prepared by an additional dilution of its stock solutions with ethanol for spectrophotometric methods.

Working solution of Nx degraded (0.03 mg mL^{-1}) was prepared by an additional dilution of their stock standard solutions with ethanol for chemometric methods.

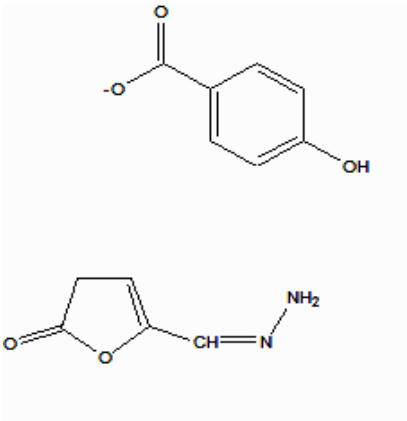

Figure 2: Alkaline induced degradation products of Nifuroxazide.

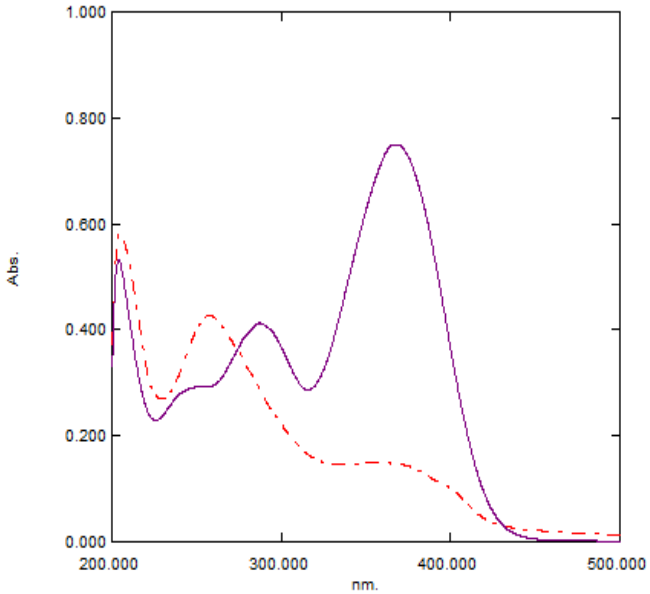

Figure 3: Absorption spectra of 8 µg.mL−1of Intact Nx (——) and 8 µg.mL−1 of degraded Nx (- - - -) in ethanol.

All the prepared solutions were protected from light.

Laboratory prepared mixtures

Solutions containing different ratios of intact and degraded forms of Nx were prepared in a set of 10 mL measuring flasks, from 1–9 µg mL^{-1} of each form and were protected from light.

Dosage form sample solutions

Five Antinal® capsules (labeled to contain 200 mg of Nx, Amon Pharmaceuticals) were accurately evacuated and weighed. An accurate weight of the powder equivalent to 10 and 20 mg of Nx were separately transferred into 100 mL volumetric flask and extracted with 60 mL ethanol, in ultrasonic bath for 30 minutes, diluted to volume with the same solvent and filtered. Suitable dilutions were made using ethanol to prepare solutions containing 10 and 20 µg mL^{-1} Nx respectively and protected from light.

Procedures

Construction of calibration curves for the ^2D spectrophotometric method

Aliquots of Nx working standard solution (0.2 mg mL^{-1}) equivalent to 2–12 µg mL^{-1} were accurately and separately transferred into a series of 10 mL volumetric flasks; the volume was completed to the mark with ethanol. The zero order absorption spectra of the prepared solutions were recorded, then the second derivative curves (2D) were obtained using $\Delta\lambda$ = 8 and scaling factor = 1000. The calibration curve was obtained by plotting the peak amplitude of 2D spectra at 278 nm (corresponding to zero crossing of degraded form) of 2D spectra versus the corresponding concentrations. The same procedure was used to determine the content of intact Nx in laboratory-prepared mixtures and in pharmaceutical formulation sample solution.

Construction of calibration curve for the ^2DD spectrophotometric method

The absorption spectra of standard solutions of Nx (2-14) µg mL^{-1} were recorded against ethanol as a blank and stored in the computer.

The stored spectra of Nx were divided by the stored spectrum of the degraded Nx in the concentration of 10 μgmL^{-1} and the second derivative of the ratio spectra (2DD) was obtained with $\Delta\lambda$ = 8 and scaling factor = 100. A calibration curve was obtained by plotting the peak amplitude at 290.8 nm versus the corresponding concentration of Nx and the regression equation was computed. The same procedure was used to determine the content of intact form of Nx in laboratory-prepared mixtures and in pharmaceutical formulation sample solution.

Chemometric methods

Multilevel multifactor experimental [15] design was applied for the construction of the calibration and validation sets. A five-level, two-factor experimental design was used in which 0.8, 0.9, 1, 1.1 or 1.2 mL aliquots of both intact and degraded form of Nx working solutions were combined and diluted to 10 mL with ethanol. The concentrations details are given in Table 1. The absorption spectra of the prepared mixtures were recorded over the wavelength range 200-500 nm and transferred to Matlab [16] for subsequent calculations.

Results and Discussion

Reviewing the literature on the determination of Nifuroxazide revealed the lack of any spectrophotometric methods for the determination of the intact drug in presence of its alkaline induced degradation products. The aim of the present work was therefore to develop simple, rapid, accurate, selective and reproducible spectrophotometric methods for the determination of intact Nx in presence of its degradation products.

No.of Mix	Intact (μg/ml)	Degraded (μg/ml)
1	10	3
2	10	2.4
3	8	2.4
4	8	3.6
5	12	2.7
6	9	3.6
7	12	3
8	10	2.7
9	9	2.7
10	9	3.3
11	11	3.6
12	12	3.3
13	11	3
14	10	3.6
15	12	3.6
16	12	2.4
17	8	3.3
18	11	2.4
19	8	3
20	10	3.3
21	11	3.3
22	11	2.7
23	9	2.4
24	8	2.7
25	9	3

Table 1: (Concentration of Intact and Degraded Nx mixtures used in chemometric methods).

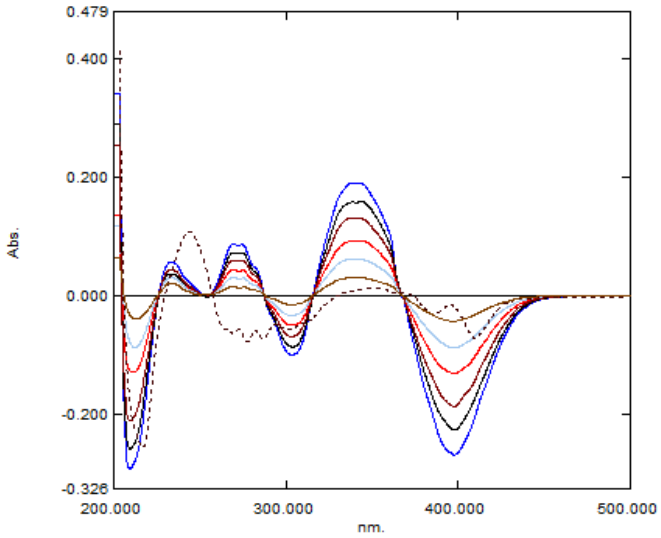

Figure 4: First derivative absorption spectra of Intact Nx (2-12 μgmL^{-1}) (——) and 10 μg.mL^{-1} of degrade Nx (------)in ethanol.

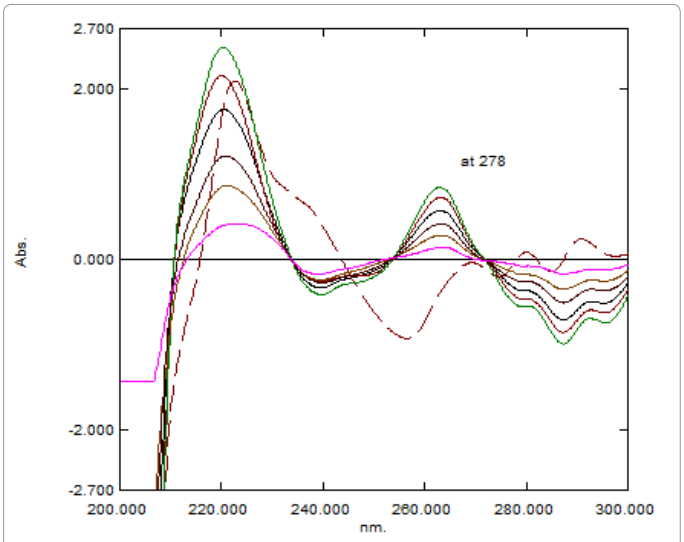

Figure 5: Second derivative absorption spectra of Intact Nx (2-12 μgmL^{-1}) (——), and 8 μgmL^{-1} of degraded Nx(- - -) in ethanol.

For 2D method

Derivative spectrophotometry is a very useful analytical technique for eliminating spectral overlapping by using the first or higher derivatives of absorbance with respect to wavelength [17]. Upon applying the derivative spectrophotometry for the determination of Nx, It was found that zero order (Figure 3) and first derivative spectra photometry (Figure 4) failed to determine Nx in presence of its degradation products. Nx could be successfully determined at about 278 nm without any interference from its degradation products, Nx could be determined by measuring its peak amplitude of 2D spectrum at 278 nm (corresponding to zero-crossing of degraded form) (Figure 5).

In order to optimize the 2D method, different smoothing and scaling factors were tested, where a smoothing factor $\Delta\lambda$ = 8 and a

scaling factor = 1000 showed a suitable signal to noise ratio and the spectra showed good resolution. A linear correlation was obtained between the peak amplitude and its corresponding concentration for Nx at λ = 278 nm in the range of 2–12 µg mL^{-1} . The parameters of the regression equations are shown in Table 2. The linear regression equations were found to be:

A = 0.0465 C – 0.0255 ,r = 0.9998

where A is the peak amplitude of 2D curves at 278 nm, C is the concentration of the drug in µg mL^{-1} and r is the correlation coefficient.

For 2DD method

In order to improve the selectivity of the analysis of Nx in presence of its degradates, derivative ratio of spectra was also applied. It was found that first derivative ratio spectra photometry failed to determine Nx in presence of its degradation products. 2DD was established and validated. The main advantage of this method is that the whole spectrum of the interfering substance is cancelled [18]. In order to optimize the 2DD method, several divisors were tested as 6, 8, 10 µg mL^{-1} absorption spectrum of Nx degradates along with its normalized curve. The best results were obtained using the spectrum of 8 µgmL−1 of the degraded form of Nx as a divisor. The absorption spectra of Nx in the range of 2-14 µg mL^{-1} were divided by the absorption spectrum of 8 µg mL^{-1} of the degraded form of Nx. The second derivatives of the obtained ratio spectra curves were then calculated using $\Delta\lambda$ = 8 and scaling factor = 100. 2DD values showed good linearity and reproducibility at 290.8 nm (Figure 6). The linear regression equation was found to be:

A = 0.0275 C + 0.0083 , r = 0.9994

where A is the peak amplitude of 2DD curves at 290.8 nm, C is the concentration of the drug in µg mL^{-1} and r is the correlation coefficient.

For chemometric methods

Multivariate calibration methods are very useful in spectral analysis because the simultaneous inclusion of many spectral wavelengths instead of using a single wavelength greatly improves the precision and predictive ability of these methods [19].

For spectral resolution of intact and degraded form of Nx, three different regression models were constructed and used for the determination of intact and degraded form of Nx in their laboratory prepared mixtures and in pharmaceutical preparation. These regression methods are CLS, PCR and PLS.

Parameters	2D method	2DDmethod
Range(µg/ml)	2-12 µg/ml	2-14 µg/ml
Slop	0.0465	0.0275
S.E. of slop	0.0004	0.0003
Intercept	-0.0255	0.0083
S.E. of Intercept	0.0032	0.0034
r	0.9998	0.9994
Mean±RSD% (accuracy)	100.36±1.836	99.81±1.889
LOD	0.2182	0.4511
LOQ	0.6612	1.3672
Repeatability %	1.471	1.863
Intermediate precision %	1.616	1.868

Table 2. (Assay validation obtained by applying the two proposed spectrophotometric methods).

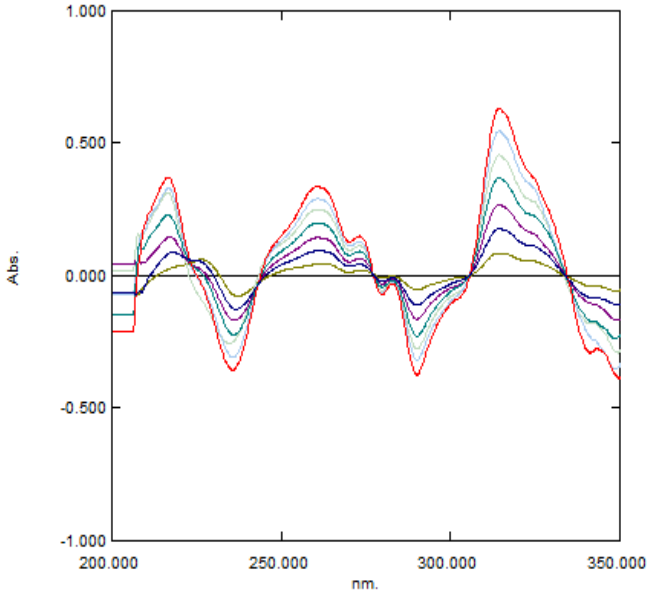

Figure 6: Second derivative of the ratio spectra of Intact Nx (2-14 µgmL−1) in ethanol.

Parameters	Chemometric methods		
	CLS	PCR	PLS
Range(µg/ml)	8-12 µg/ml	8-12 µg/ml	8-12 µg/ml
Slop	0.9917	0.9921	0.9929
S.E. of slop	0.0498	0.0490	0.0491
Intercept	0.1129	0.0987	0.0966
S.E. of Intercept	0.5038	0.4999	0.4968
r	0.9992	0.9994	0.9998
LOD	0.45	0.44	0.45
LOQ	1.37	1.35	1.36
Precision	1.13-1.21	1.13-1.21	1.02-1.01

Table 3: (Assay validation obtained by applying the proposed chemometric methods).

Experimental design of the calibration and validation sets

Brereton [15] constructed multilevel-multifactor design in which, the levels (L) are the concentrations used and the number of experiments is L2. For the calibration and validation sets, different laboratory prepared mixtures of intact and degraded form of Nx were prepared. The concentration range for intact form is 8.0-12.0 µg mL^{-1}, Table 1, and for degraded form the range is 2.4-3.6 µg mL^{-1}. The spectra of the prepared mixtures were recorded in the range of 200-500 nm and the spectral data acquisition was taken with 0.1nm intervals, thus producing 1001data points per spectrum. To build up the spectral data matrix the spectral data acquisition was taken every 1 nm. In order to decrease the initial number of wavelengths; every tenth wavelength was selected, thus the produced spectral data matrix has 25 rows representing different samples and 101 columns representing wavelengths (25 x 101). Seventeen samples were chosen and used for calibration and eight were used for external validation.

CLS model

The training set was used for constructing CLS model or (K) matrix (i.e. absorptivity at different wavelengths). The CLS method requires

validation Mix.	R% of Nx			R% of Nx degradates		
	CLS	PCR	PLS	CLS	PCR	PLS
1	100.76	99.23	99.11	101.49	99.07	98.98
2	97.81	99.36	99.26	109.11	96.20	97.88
3	97.39	96.56	98.60	114.36	118.40	104.96
4	96.55	97.88	97.92	112.83	99.63	98.59
5	103.09	99.44	99.52	81.64	100.35	100.35
6	102.90	98.11	98.23	78.46	105.61	104.68
7	100.51	99.65	99.97	87.61	103.01	102.08
8	96.69	101.71	101.66	136.53	91.99	95.19
Mean	99.46	99.00	99.27	102.75	101.78	100.34
RMSEP	0.252	0.234	0.231	0.547	0.208	0.161

Table 4: (Results obtained by applying the proposed chemometric methods to the analysis of the validation set).

Method			2D		2DD	
Sample No.	%Deg.	St.conc (µg/ml)	calc.conc (µg/ml)	R%	calc.conc (µg/ml)	R%
1	10%	9	9.09	101.00	8.79	97.67
2	20%	8	8.01	100.13	7.84	98.00
3	30%	7	6.89	98.43	6.93	99.00
4	40%	6	6.05	100.83	5.95	99.17
5	50%	5	5.09	101.80	5.04	100.80
6	60%	4	3.99	99.75	3.99	99.75
7	70%	3	3.06	102.00	2.93	97.67
8	80%	2	2.05	102.50	1.80*	90.36*
Mean±SD				100.80±1.339		98.87±1.172
RSD%				1.328		1.173

* Not included in calculations.

Table 5: (Determination of Nifuroxazide in laboratory prepared mixtures by applying the proposed methods 2D, 2DD).

method	2D method				2DD method			
Pharmaceutical Dosage Form	Dosage Form	Standard addition			Dosage Form	Standard addition		
	Found %*±RSD%	Pure added (µg/ml)	Found (µg/ml)	R%	Found %*±RSD%	Pure added (µg/ml)	Found (µg/ml)	R%
Antinal Capsules (batch no.2488)	101.22±1.261	4	4.05	101.25	101.69±1.877	4	4.03	102.75
		6	5.99	99.83		6	6.03	100.50
		8	7.86	98.25		8	8.10	101.25
Mean±RSD				99.77±1.504				101.50±1.145

* Average of three determinations.

Table 6: (Determination of Nifuroxazide in Antinal® capsules by the proposed 2D, 2DD methods and application of standard addition techniques).

that all the components in the calibration samples must be known. Unlike CLS, PCR and PLS methods could be used to determine the components under investigation even in the presence of unknown components (interfering substance) which gave these two methods an advantage over CLS [20]. The absorbance matrix of the calibration samples (17x101) and their corresponding concentration matrix (17x2) were used to find the absorptivity matrix (k-matrix). Then, the obtained k-matrix was further used for the calculation of the predicted concentration of the two components in both the validation and pharmaceutical formulation samples.

PCR and PLS models

In order to apply PCR and PLS to the data, the raw data of the calibration samples were mean centered [21] as a preprocessing step and random subsets was applied as an internal cross validation method [22]. To choose the optimum number of significant latent variables, F statistics [23] was applied. After the PCR and PLS models have been constructed, it was found that the optimum number of LVs described by the developed models was four and two factors for PCR and PLS, respectively.

Calibration graphs were constructed by plotting the predicted concentrations for each compound by each of the developed models versus the true concentrations. The statistical parameters of the linear relationship between the calculated and the true concentration of Nx in the calibration set are represented in Table 3. In order to assess the predictive ability of each of the developed models, it was applied on an external validation set for determination of the two components. The recoveries, mean recoveries, standard deviation, precision and RMSEP

Chemometric methods			
	CLS	PCR	PLS
Antinal Capsules	100.46±3.79	99.78±1.27	100.15±1.09
Standard Addition	100.02±2.32	99.99±1.23	99.87±0.98

Table 7: (Determination of Nifuroxazide in Antinal® capsules by the proposed chemometric methods and application of standard addition techniques).

Parameter	2D method	2DD method	Chemometric methods			manfacturer method(b)
			CLS	PCR	PLS	
Mean	99.97	99.83	99.46	99.00	99.77	99.76
SD	1.50	1.40	2.69	1.51	1.32	1.32
N	6	7	8	8	8	5
Variance	2.26	1.96	7.24	2.28	1.75	1.75
Student's t	0.24(2.26)a	0.08(2.26)a	0.26(1.79)	0.96(1.81)	0.69(1.85)	
F	1.29(6.25)a	1.12(6.16)a	4.15(6.09)	1.31(6.09)	1.30(4.12)	
a These values represent the corresponding tabulated values of t & F at P=0.05						
b spectrophotometric method						

Table 8: (Statistical analysis of the results obtained by applying the proposed methods and reported method for the analysis of pure powder Nx).

values are summarized in Tables 3 and 4. It is clear from the obtained results that the PLS model is the most efficient model regarding its predictive ability and described by two factors representing the two components present in the laboratory prepared mixtures, so, this model was applied for the determination of Nx in pharmaceutical dosage form.

The specificity of the proposed methods was proved by the analysis of laboratory-prepared mixtures of intact Nx in different ratios, as presented in Tables 4 and 5. All the proposed methods were successfully applied for the determination of Nx in Antinal capsules, Tables 6 and 7.

The results obtained were statistically compared with those obtained by the reported spectro method and there is no significant difference regarding both accuracy and precision as shown in Table 8.

Conclusion

The proposed methods were simple, rapid, sensitive and precise and could be easily applied in quality-control laboratories for the determination of intact Nx in presence of its alkaline induced degradates. So the proposed methods could be successfully applied for the routine analysis of this drug either in its pure bulk powders or in dosage form in quality-control laboratories without any preliminary separation step.

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