December 2009 Volume 8 Issue 4

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 8(4) 2009 [613-620]

Stability indicating methods for determination of nafronyl oxalate

Samah S.Abbas, Hala E.Zaazaa, Mohamed R.El-Ghobashy, Yasmin M.Fayez*, Soheir A.Fattah

Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini St., 11562, Cairo, (EGYPT)

E-mail : yasminfayez@maktoob.com

Received: 28th August, 2009 ; Accepted: 7th September, 2009

ABSTRACT

HPLC method with spectroscopic detection for determination of Nafronyl (Naftidrofury)l Oxalate (NFL), a rapid separation could be achieved by a C_{18} column using mobile phases of 72%methanol-1% triethylamine -0.6% phosphoric acid. The eluate was monitored at 282.4. Two chemometric techniques were applied for determination of NFL namely partial least squares (PLS-1) and genetic algorithm based wavelength selection- partial least squares (GA-PLS) regression methods. The suggested methods were used to determine NFL in synthetic mixtures and in commercial tablets. The validity of the proposed methods was further assessed by applying standard addition technique. The obtained results were statistically compared with official method, showing no significant difference with respect to accuracy and precision.

KEYWORDS

Naftidrofuryl Oxalate;
Nafronyl Oxalate;
Praxilane;
High Performance Liquid Chromatography;
Genetic algorithm.

INTRODUCTION

Naftidrofuryl Oxalate is Tetrahydro-α-(1-naphthalenylmethyl)-2-furanpropanoic acid 2-(diethylamino) ethyl ester acid oxalate[1] as in Figure 1.

Several chromatographic methods have been recommended for the determination of NFL; these include titrimetric method[1], HPLC[2-5], Capillary zone electrophoresis[6], electrochemical method[7] and Phosphorimetry[8-12].

The NFL is an official in any pharmacopoeia. It is desirable to develop a simple and fast procedure that could be applied in quality control laboratories for the selective determination of NFL in the presence of its alkaline degradation product. The utility of the developed methods to determine the content of drug in its pharmaceutical dosage form is also demonstrated.

EXPERIMENTAL

Instruments

1- Schimadzu instrument, equipped with a variable wavelength detector and 20-µL volume injection loop.

2- Column of shim. Pack shimadzu VP-ODS C_{18} (4.6 x 150 mm I.D.).

3- A double beam UV-Visible spectrophotometer (SHIMADZU, Japan) model UV-1601 PC with quartz cells of 1cm path length, connected to IBM compatible computer and HP 680 inkjet printer. The bundled software was UVPC personal spectroscopy software version 3.7. The spectra bandwidth was 2nm and wavelength – scanning speed 2800 nm/min.

4- Ultrasonic, Bandelin electronic, Sonorex RK51OS, HF-Frequency 35 KHz (10 liter capacity), Fed-

Full Paper

eral republic of Germany.

5- Potentiometric measurements were made at 30 ±1°C with a Hanna (Model 211) pH/mV meter. A single junction calomel reference electrode (Model HI 5412) was used in conjunction with the drug sensor. A WPA pH combined glass electrode Model CD 740 was used for pH measurements.

Reagents and solvents

All reagents used throughout this work were of analytical pure grade, and solvents were of spectroscopic grade.

1. Methanol, water, chloroform, triethylamine, phosphoric acid and water (HPLC grade).
2. NaOH (ADWIC) - El Nasr Pharmaceutical & Chemical Co. Egypt 1N NaOH.

Samples

Pure standard

NFL was kindly supplied by MINAPHARM-Egypt under license of MERK France having a purity of 100.28 ± 1.119 according to the official method[1].

Pharmaceutical dosage form

Praxilene® film coated tablets (MINAPHARM-Egypt under license of MERK France) labeled to contain 200mg of NFL per tablet, batch number 6IE1312.

Degraded sample

Preparation of its alkaline degradation product of NFL

It was prepared by dissolving 25mg of NFL in 9mL water, and then 10ml methanol was added followed by 4 mL of 1N NaOH and refluxing for 4 hours. The solution was neutralized, evaporated to dryness on hot plate and then the residue was dissolved in 20mL methanol. The obtained solution was filtered into 25-ml volumetric flask and the volume was completed with methanol to have a concentration of 1 mg mL^{-1}.

Standard stock and working solutions

a- Standard stock solution of NFL and its alkaline degradation product (1 mg mL^{-1}) of each.

b- Working standard solution of NFL and its alkaline degradation product (100 µg mL^{-1}) of each.

c- Working standard solution of NFL and its alkaline degradation product (200 µg mL^{-1}) of each.

d- Laboratory prepared mixtures of in different ratios from 10 to 90%

Procedure

For HPLC method

Chromatographic conditions

Record the chromatograms of NFL and its its alkaline degradation product using a shimadzu instrument operated under the following parameters:

- Flow rate: 1mL/min. at ambient temperature.
- Detector: adjusted at 283 nm.
- Column: Schimadzu ODS-C18 (4.6 X 150MM I.D.).
- Mobile phase: 72% methanol: 1% triethylamine: 0.6% phosphoric acid. (The mobile phase was filtered using 0.45 µm membrane filter and degassed by ultrasonic vibrations for 10 min).
- Result output: chromatogram and peak area.

Construction of calibration graph for determination of NFL by HPLC method

Aliquots of NFL working standard solution (100 µg mL^{-1}) equivalent to 100-600 µg were accurately transferred into a series of 10-ml volumetric flasks, the volume was completed to the mark with the mobile phase.

Analyze the prepared samples using the previously mentioned chromatographic conditions and record the peak area then construct a calibration curve correlating the peak areas of NFL to the corresponding concentrations. Compute the corresponding regression equations.

Determination of NFL in presence of its its alkaline degradation productn laboratory prepared mixtures by the proposed methods

Aliquots of 6 to 1 ml were separately transferred from NFL working standard solution (100µg mL^{-1}) into 10-ml volumetric flasks. To the previous solutions, aliquots of 1 to 9 ml of I and II working standard solutions (100 µg mL^{-1}) were added separately and the volume was completed to the mark with mobile phase. Mixtures of different ratios were obtained and the peak areas at 282.4 nm were measured in presence of II. Using the procedure described under 2.5.2, the concentration of the intact drug was calculated from its corresponding regression equations.

For Genetic algorithm

Construction of the training set

Prepare different mixtures of NFL and its alkaline degradation product by transferring different volumes of NFL and its alkaline degradation product stock solution into a series of 25-ml volumetric flasks.

Complete the volume with methanol and scan the absorbance of these mixtures between 200 and 400 nm with respect to a blank of methanol.

Pre-processing the data

Reject the regions from 200-230 nm and above 320nm. Mean centering of the data was performed.

Validation

External validation set of 13 samples was prepared to check the performance of the constructed model.

Determination of NFL in pharmaceutical dosage form by the proposed method

Film coat of ten tablets were removed using a filter paper moistened with methanol, and a weight equivalent to 100 mg of NFL dissolved in 20 mL methanol, sonicated for 8 min, mixed well and then filtered. The general procedures were followed.

RESULTS AND DISCUSSION

The stability of NFL was studied according to the ICH guidelines for:

Stress, acid and alkaline

Reflux with 0.1N HCl /0.1N NaOH for 8 hours, 1N HCl for 12 hours, 2N HCl for 24 hours finally 6N HCl for 24hours.

Oxidative condition

Reflux with 3% H_2O_2 for 24 hours and 10% for 24 hours.

The degradation process under the previously mentioned conditions was followed using TLC and the compound was found to be stable under acidic condition but it is liable to degradation in alkaline condition giving one component which is confirmed with a previous study on stability of NFL[3].

To detect the complete degradation of NFL, a TLC procedure was suggested. Different systems were tried, where complete separation of NFL from its alkaline degradation product was achieved using methanol-chloroform (20-80 by volume) as the mobile phase. The R_f values were 0.79 for NFL and 0.71for degradate me.

Using other systems such as methanol-chloroform-ammonia and butanol-chloroform-ammonia,butanol-ethyl acetate ammonia, methanol-ethyl acetate-ammonia and methanol-ethyl acetate-acetic acid in different ratios were not efficient for separating NFL from its alkaline degradation product, except methanol-ethyl acetate-ammonia in the ratio (5-5-0.1 by volume) and methanol-chloroform in the ratio (2-8 by volume),the second system was used from the economic point of view. Spotting of 5µg at different successive times of reflux and after evaporation, showed complete alkaline degradation after four hours and the obtained degradate unaffected during evaporation. Under alkaline–induced degradation condition; one component was produced as indicated by the appearance of one spot of its alkaline degradation product after complete degradation that also confirmed by IR.

A suggested structures for alkaline degradates as in **Figure 2**[3].

Elucidation of IR spectroscopy of the degradate confirm its structure; where the oxalate group in the drug (that appeared at 1636.43 cm^{-1}) was disappeared and a new carboxylate group corresponding to the its alkaline degradation product was appeared at1729.94 cm^{-1}.Also, (C=O) and (OH) of the free carboxylic acid appears at 1573.16 and 3326.24 cm^{-1}, respectively as in Figures 3-5.

Upon scanning the absorption spectra of each of NFL and its alkaline degradation product, it was observed that NFL has a λ_{max} at 272.6 and 282.6nm, at this wavelengths, its alkaline degradation product show complete overlapping as shown in Figure 6. Thus unable to use of zero-order absorption spectra for the selective determination of the drug.

This work concerned with the determination of NFL in presence of its degradate.

For HPLC method

This work concerned with the determination of NFL in presence of its degradate by applying HPLC technique.

Full Paper

Different mobile phases were tried for the chromatographic separation of the components and the best resolution was achieved using a mobile phase composed of 72%methanol-1% triethylamine-0.6% phosphoric acid. Using the specific chromatographic conditions retention times were found to be 2.95 and 4.90 for NFL and its alkaline degradation product, respectively.

Statistical analysis of the parameters required for system suitability test of HPLC method indicates good resolution of the two components as in TABLE 1.

TABLE 1 : Parameters required for the system suitability test of the HPLC method

Parameter	Obtained value	Reference values[1]
Resolution	2.37	R > 1.5
T(tailing factor)	1.25	T=1 for a typical symmetric peak
A(relative retention time)	1.68	>1
K(column capacity)	1.095	1-10acceptable
N(column efficiency)	1301	Increases with efficiency of the separation
HETP=L(length of column in cm)/N	0.049	The smaller the value, the higher the column efficiency

A linear relationship was obtained between the peak area at wavelength 282nm and the corresponding concentration.

The regression equation was calculated and found to be:

A = 11801.4957C + 46477.4000 r = 0.9999

Where, "A" is the peak area, "C" is the concentration in µg mL^{-1} and "r" is the regression coefficient.

The mean percentage recoveries and standard deviations of the pure drug were calculated as shown in TABLE 2.

TABLE 2 : Determination of pure NFL by the HPLC method.

Taken (µg.mL^{-1})	Found (µg.mL^{-1})	Recovery%
10.00	10.21	102.10
20.00	19.78	98.90
30.00	29.87	99.57
40.00	40.15	100.38
50.00	49.90	99.80
60.00	60.09	100.15
Mean ± S.D		100.15 ± 1.084

The results shown in TABLE 3 contributed to the good performance of the methods with high selectivity to determine the studied drug in presence of its alkaline degradation product.

TABLE 3 : Results of laboratory prepared mixtures of NFL in presence of its its alkaline degradation product by the HPLC method

% degradation product	Recovery % of NFL
16	98.51
33	100.83
50	99.12
67	96.74
83	100.08
Mean ± SD	99.06 ± 1.570

The proposed methods were also applied for the determination of NFL in its dosage form. Furthermore, the validity of the methods were assessed by applying the standard addition technique, as in TABLE 4, mean percentage recovery revealed that there was no interference from any excipients, that may be found in the pharmaceutical dosage forms.

TABLE 4 : Determination of NFL in Praxilane® film coated tablets by the HPLC method and application of standard addition technique

Method	Praxilane® 200 mg NFL/tablet (B.N:7HE1282)	Standard addition technique	
	Recovery%* ± S.D	Pure added (µg.mL^{-1})	Recovery %
HPLC	99.39 ± 1.551	20	98.13
		30	97.30
		40	98.96
		Mean ± S.D	98.13 ±0.830
PLS1	105.32 ± 2.800	10	101.28
		20	102.11
		30	101.24
		Mean ± S.D	101.54 ±0.491
GA-PLS1	98.94 ± 2.489	10	96.34
		20	96.48
		30	97.34
		Mean ± S.D	96.72 ±0.541

Method validation was performed according to USP 2007[13] for the proposed method as in TABLE 5.

TABLE 5 : Validation results of the HPLC method for the determination of NFL

Parameters	[1]DD
Linearity	
Slope	11801
Intercept	46477
Correlation coefficient	0.9999
Range	10.00-90.00 μg.mL^{-1}
Accuracy (Mean±S.D)	100.15 ± 1.079
Precision (RSD %)	
Repeatability[(a)]	1.700
Intermediate precision[(b)]	1.380

(a)The intraday (n=3), average of three different concentrations repeated three time within the day.
(b) The intermediate precision (n=3), average of three different concentrations repeated three times in three days.

For PLS-1 Method

The quality of multi component analysis is dependent on the wavelength range and spectral mode used[14].

PLS procedures are designated to be full spectrum computational procedures; however, using highly noisy, scarcely informative wavelengths detracts from precision. This can be lessened by discarding particularly noisy wavelengths. The wavelengths used were in range 230-320 nm in all cases. Wavelengths less than 230 nm were rejected due to the noisy content. Wavelengths more than 320 nm were not used because corresponding components do not absorb in these regions.

The multivariate calibration requires a careful experimental design of the standard composition of calibration set for providing the best predictions. Multilevel multi factorial design[15] was used for the construction of the calibration set. A calibration set consisting of 9 samples was used.

The concentrations of NFL and its alkaline degradation product were varied over the range 40-60 μg.ml^{-1} for both in the calibration set. TABLE 6 shows the composition of the calibration set. PLs-1 method was run on the calibration data of absorption UV spectra.

TABLE 6 : The concentration of different mixtures of NFL and its alkaline degradation product used in the training set

Mix. No.	Mix. composition	
	NFL	Its alkaline degradation product
1	45.2	50
2	60	45.2
3	45.2	54.8
4	45.2	60
5	54.8	54.8
6	60	50
7	50	60
8	60	40
9	40	54.8

To select the number of factors in the PLS-1 algorithm, a cross-validation method leaving out one sample at a time[16] was employed using calibration set of 9 calibration spectra. PLS-1 calibration on 8 calibration spectra was performed and, using this calibration, the concentration of the sample left out during the calibration process was predicted. This process was repeated 9 times until each training sample had been left out once. The predicted concentrations of the components in each sample were compared with the actual concentrations in this calibration samples and the root mean squares error of cross-validation (RMSECV) was calculated as follows:

$$\mathbf{RMSECV} = \sqrt{\frac{\mathbf{PRESS}}{\mathbf{n}}}$$

Where n is the number of calibration samples.

$$\mathbf{PRESS} = \Sigma\,(\mathbf{Y_{pred}} - \mathbf{Y_{true}})^2$$

Where Y_{pred} and Ytrue are predicted and true concentrations in μg.ml-1, respectively.

The RMSECV was used as a diagnostic test for examining the errors in the predicted concentrations. It indicates both of the precision and accuracy of predictions. It was recalculated upon addition of each new factor to the PLS-1 model. Appropriate selection of the number of factors to be used to construct the model is a key to achieving correct quantitation in PLS-1 calibration. The most usual procedure for this purpose involves choosing the number of factors that result in the minimum RMSECV. However, this criterion is subjected to some constraints since, oc-

casionally; the RMSECV does not reach a sharp minimum, but decreases gradually above a given number of factors. For this reason, the method developed by Haaland Thomas[17] was used for selecting the optimum number of factors, which involves selecting that model including the smallest number of factors that results in an insignificant difference between the corresponding RMSECV and the minimum RMSECV.

Number of factors used for each drug is shown in TABLE 7. As the difference between the minimum RMSECV and other RMSECV.

TABLE 7 : Statistical parameter values for simultaneous determination of NFL using optimized PLS-1 and GA-PLS methods

Parameter of interest	PLS-1 method	GA-PLS method
Concentration range ($\mu g.ml^{-1}$)	(40-60)	(40-60)
No. of factors	7	6
RMSECV	2.694	1.358
Intercept	-3.4	2.2738
Slope	1.0736	0.9536
Correlation coefficient (r)	0.9994	0.9998

Values become smaller; the probability that each additional factor is significant becomes smaller[18]. The selected model is that with the smallest number of factors such that RMSECV for that model is not significant greater than RMSECV from the model with additional factor.

In order to validate proposed PLS-1 method, a validation set composed of 13 synthetic mixtures was analyzed with the proposed PLS-1 method.

PLS-1 method was run on the calibration data using optimal number of latent variables. The concentrations of NFL in calibration set were calculated as shown in TABLE 6. By plotting predicted concentrations of each component versus actual concentrations, a straight line is obtained. The data of the straight line for each component including slope, intercept and correlation coefficient is collected in TABLE 7.

PLS-1 method was run on the validation set using optimal number of factors and predicted concentration of NFL is given in TABLE 8.

TABLE 8 : Results of the analysis of the mixtures of the validation set of NFL by the proposed method

sample no.	concentration of NFL ($\mu g.ml^{-1}$)	PLS-1	GA
1	50	98.41	97.06
2	50	101.52	99.40
3	40	101.16	97.70
4	40	98.04	97.96
5	60	105.56	104.75
6	54.8	98.16	101.32
7	60	98.73	97.41
8	40	103.26	99.16
9	50	99.81	101.02
10	54.8	100.11	100.79
11	54.8	97.42	98.34
12	45.2	101.15	101.01
13	40	102.46	101.05
Mean		100.44	99.77
S.D		2.382	2.160
RSD		2.371	2.165

GA-PLS method

Constructing the PLS model after selecting the optimal variables (wavelengths) improves the prediction capacity of the model[19]. GA can be used successfully for wavelength selection in PLS calibration. GA for wavelength selection consists of five steps (as mentioned in the introduction): representation, initiation, evaluation, genetic operators and genetic parameters. A critical issue of successful GA performance is the adjustment of GA parameters. The parameters allowed for adjustment in PLS-Toolbox are: the maximum number of generations, the number of wavelengths in a window, percent genes included at initiation, the mutation rate, breeding cross over rule and percent of population the same at convergence.

Other parameters to be chosen by the user are: maximum number of latent variables for the PLS, cross validation type random or contiguous blocks, number of subsets to divide data into for cross validation, number of interactions for cross validation at each generation. The configuration of GA parameters is shown in TABLE 9.

Each solution (chromosome) is evaluated using the PRESS value reached in the calibration. The GA

TABLE 9 : Parameters of the genetic algorithms

Parameter	Value
Population size	256
Maximum generations	400
Mutation rate	0.005
The number of variables in a window (window width)	5
Percent of population the same at convergence	80
% wavelengths used at initiation	50
Crossover type	2 (double)
Maximum number of latent variables	4
cross validation option	0 (random)
Number of subsets to divide data into for cross validation	5
Number of iterations for cross validation at each generation	1

searches for the minimum PRESS in the space of all the possible chromosomes without establishing, a prioi, and the latent structure of the calibration. The GA was run for 91variables (in the range 230-320 nm) for NFL using a PLS regression method with maximum number of factors allowed is the optimal number of components determined by cross-validation on the model containing all the variables, and the selected variables were used for running of PLS.

Figure 7 show the frequency with which each variable (wavelength) was preselected for NFL. For obtaining the optimum set of wavelengths for determination of NFL, the GA procedure was repeated 10 times. Finally a wave length was selected if the percent of selection for that variable exceeds a critical value. The thresholds of 80% were obtained for NFL, according to minimum error of prediction for each analyte.

GA reduced the optimal number of factors for NFL as shown in TABLE 7 also RMSECV was slightly decreased indicating an increase in power of prediction of GA-PLS model than PLS model. The predictive ability of the method was further checked on validation set, the results are shown in TABLE 8. The comparison of GA-PLS results with PLS-1 shows, the GA-PLS is more suitable for simultaneous determination of NFL.

The proposed methods were also applied for the determination of NFL in its dosage form as shown in TABLE 4.

The results obtained by applying the proposed methods were statistically compared with the official method[1] and no significant difference was found regarding accuracy and precision as in TABLE 10.

TABLE 10 : Statistical analysis of the results of the proposed methods and the official method for determination of pure NFL

ITEMS	HPLC	PLS-1	GA-PLS	Official method*[1]
Mean ± S.D	100.15 ± 1.084	100.44 ± 2.381	99.77 ± 2.160	100.28 ± 1.119
%RSD	1.082	2.371	2.165	1.116
n	6	13	13	6
Variance	1.175	5.669	4.666	1.203
Student's t test	0.204 (2.228)	0.42 (2.101)	0.74 (2.101)	
F value	1.02 (5.05)	4.53 (4.72)	3.73 (4.72)	

***Non-aqueous titration with 0.1 M perchloric acid, determining the end point potentiometrically.**
- Figures in the parenthesis are the corresponding theoretical values of t and F at 0.05 level of significance

CONCLUSION

Two methods were presented for the analysis of NFL in pure powder and in Praxilane tablets. The first method is PLS-1 using full spectrum and it was suitable for determination of NFL in presence of its its alkaline degradation product.

The second method was GA-PLS in which GA function found in PLS-Toolbox was used as a way of wavelength selection. GA-PLS had an advantage of reducing variables and so increasing predictive ability of PLS model.

The suggested methods are simple, fast and can be used for routine and quality control analysis of NFL in raw material and in pharmaceutical preparation without interferences of excipients.

REFERENCES

[1] 'British Pharmacopoeia', Her Majesty's Stationary Office, London, **(2004)**.

[2] R.R.Brodie, L.F.Chasseaud, T.Taylor, J.O.Hunter, P.J.Ciclitira; J.Chromatogr.B Analyt.Technol. Biomed.Life Sci., **164**, 534 **(1979)**.

[3] E.R.Garrett, R.Barbhaiya; J.Pharm.Sci., **70**, 39 **(1981)**.

[4] L.M.Walmsley, P.A.Wilkinson, R.R.Brodie, L.F.Chasseaud; J.Chromatogr., **338**, 433 **(1985)**.

Full Paper

[5] P.Stehlik, H.Houbova; Cesk Farm, **39**, 394 **(1990)**.
[6] F.Wang, T.Dowling, G.Bicker, J.Wyvratt; J.Sep.Sci., **24**, 378 **(2001)**.
[7] M.S.Ionescu, V.Badea, G.E.Baiulescu, W.Cosofret; Talanta, **33**, 101 **(1986)**.
[8] Munoz-de-la-Pena, Espinosa-Mansilla, Murillo-Pulgarin, Alanon-Molina, Fernandez-Lopez; Analyst, **123**, 2285 **(1998)**.
[9] Murillo-Pulgarin, Alanon-Molina, Fernandez-Lopez; Analytica-Chimica-Acta., **382**, 77 **(1999)**.
[10] Segura-Carretero, Cruces-Blanco, Canabate-Diaz, Fernandez-Sanchez, Fernandez-Gutierrez; Analytica-Chimica-Acta., **417**, 19 **(2000)**.
[11] Cruces-Blanco, Segura-Carretero, Fernandez-Sanchez, Fernandez-Gutierrez; J-Pharm-Biomed-Anal., **23**, 845 **(2000)**.
[12] W.Li, L.X.Cao, G.R.Li, W.J.Jin; Guangpuxue-Yu-Guangpu-Fenxi, **22**, 518 **(2002)**.
[13] 'The United States Pharmacopeia and National Formulary', The Twenty Seventh Revision, The National For Official Compendia of Standards, Asian Editionmulary, USP30-NF25,The United States Pharmacopeial Convention, Inc., Rockville, MD, **(2007)**.
[14] M.Blanco, J.Coello, F.Gonzalez, H.Iturriaga, S.Maspoch; J.Pharm.Sci., **82**, 834 **(1993)**.
[15] R.Brereton; Analyst, **122**, 1521 **(1997)**.
[16] A.Espinosa-Mansilla, F.Salinas, I.De Orbe Paya; Anal.Chim.Acta, **313**, 103 **(1995)**.
[17] D.M.Haland, E.V.Thomas; Anal.Chem., **60**, 1193 **(1988)**.
[18] N.A.El-Ragehy, S.S.Abbas, S.Z.El-Khateeb; Anal.Chim.Acta, **461**, 155 **(2002)**.
[19] R.Leardi, M.B.Seasholtz, R.J.Pell; Anal.Chim.Acta, **461**, 189 **(2002)**.