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Stability indicating methods for determination of Naftidrofuryl Oxalate

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ABSTRACT

First derivative ratio and densitometry were used to determine Naftidrofuryl Oxalate (NFL) in presence of its alkaline degradate (I). For first derivative ratio, the peak amplitude at 249.2 nm was used for selective determination of NFL in presence of I in the range of 10-90 µg mL-1, while utilizing densitometric technique allows selective determination of NFL in presence of I in range of 0.6-8 µg spot-1. 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 HPLC method, showing no significant difference with respect to accuracy and precision.

KEYWORDS

Naftidrofuryl oxalate;
First derivative ratio spectrophotometry;
Thin layer chromatography and densitometry.

INTRODUCTION

Naftidrofuryl Oxalate is Tetrahydro-α-(1-naphthalenylmethyl)-2-furanpropanoic acid 2-(diethylamino) ethyl ester acid oxalate as shown in Figure 1[1]. Several 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 drug in BP. 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 degradate. The utility of the developed methods to determine the content of drug in its pharmaceutical dosage form is also demonstrated.

1. The two suggested methods can be used as stability indicating methods to determine NFL in presence of its alkaline degradate.
2. DD1 spectrophotometric method provided simplicity and rapidity than other methods.
3. The suggested techniques are selective for determination of NFL in presence of its alkaline degradate, the most selective methods were densitometric as it can determine NFL in presence of up to 90% of its alkaline degradate.
4. The suggested methods are simple, rapid and

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from the economical point of view, all analytical reagents used are inexpensive, have excellent shelf life and available in any analytical laboratory.

5. Statistical analysis of the results obtained by the proposed methods and the reported method, showing no significant difference between them.

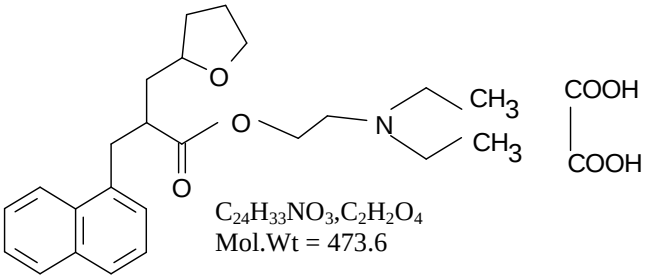

Figure 1 : Structure of Naftidrofuryl Oxalate.

EXPERIMENTAL

Instruments

1. A double beam UV-Visible spectrophotometer (SHIMADZU, Japan) model UV-1601 PC with quartz cell of 1cm pathlength, 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.
2. CAMAG TLC Scanner "Scanner 3_130319 (1.14.26).
3. TLC glass plates precoated with silica gel 60 F254 (20x20), 0.2 mm thickness (E.Merck), Darmstadt, Germany.
4. 25- µl Hamilton syringe.
5. Chromatographic tank 20 x 21 x 9 cm (Desaga)
6. UV short wavelength (254 nm) lamp. (Desaga, Germany)
7. Ultrasonic, Bandelin electronic, Sonorex RK51OS, HF-Frequency 35 KHz (10 liter capacity), Germany.

Reagents and solvents

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

1. Methanol, water, chloroform (HPLC grade).
2. NaOH (ADWIC) - El Nasr Pharmaceutical & Chemical Co.Egypt, 1N NaOH.
3. Sodium hydroxide, 0.05 M aqueous solution; Prolabo.
4. Hydrochloric acid, 0.05 M aqueous solution; Prolabo.

Samples

Pure standard

NFL was kindly supplied by MINAPHARM-Egypt under license of MERK France, having a purity of 100.28 ± 1.119[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 alkaline degradate of NFL

It was prepared by dissolving 25mg of NFL in 9mL water, 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 alkaline degradate (1 mg mL-1) of each.
b. Working standard solution of NFL and alkaline degradate (100 µg mL-1) of each.
c. Laboratory prepared mixtures of in different ratios from 10 to 90%

Procedures

Construction of calibration graph for determination of NFL by first derivative ratio method.

Aliquots of NFL working standard solution equivalent to 100-900 µg were accurately transferred into a series of 10-ml volumetric flasks, the volume was completed to the mark with methanol.

Aliquots of 9.0 ml of I working standard solution (100 µg mL-1) were accurately transferred, separately into 10-ml volumetric flask and the

volume was completed to the mark with methanol. The absorbance of each solution was measured in the range of 200-400 nm in presence of I. For the determination of NFL in presence of I, the stored spectra of NFL were divided separately by the spectrum of I. The first derivative corresponding to each ratio spectrum 1DD was recorded using $\Delta\lambda$ 4nm and scaling factor 100.

A calibration curve was constructed representing the peak amplitude of 1DD at 249.2 nm versus concentration and the regression equations were computed.

Construction of calibration graph for determination of NFL by TLC-densitometric method

Aliquots equivalent to 0.3-4 mg NFL from stock standard solution (1mg mL-1) was transferred into 10-ml volumetric flasks and the volume was completed with methanol. 20 µl was applied to thin layer chromatographic plates (20x20) using 25µl Hamilton syringe. Spots were spaced 2 cm apart from each other and 1.5cm from the bottom edge of the plate. The plates were developed in the chromatographic tank previously saturated with the developing mobile phase, chloroform: methanol (80:20 by volume), for at least 20 minutes. The plates were developed by ascending technique to a distance of about 8 cm, dried at room temperature. The spots were detected under UV-lamp and scanned, at 282nm for NFL. The peak areas were recorded for the drug. The calibration curve was constructed by plotting the area under the peak versus the corresponding concentrations of NFL. The corresponding regression equation was computed.

Determination of NFL in presence of its degradate in laboratory prepared mixtures by the proposed methods

First derivative ratio method

Aliquots of 9 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 working standard solutions (100 µg mL-1) were added separately and the volume was completed to the mark with methanol. Mixtures of different ratios were obtained and the peak amplitude of 1DD at 249.2 nm was measured in presence of I. Using the procedure described under 2.5.2, the concentration of the intact drug was calculated from its corresponding regression equations.

TLC-densitometric method

Aliquots of 0.6 to 1.7 ml were separately transferred from NFL standard stock solution (1 mg mL-1) into 10-ml volumetric flasks. To the previous solutions, aliquots of 1.7 to 0.3 ml of I working standard solutions (1mg mL-1) were added and the volume was completed to the mark with methanol. Mixtures of different ratios were obtained. The peak areas of the obtained chromatogram were measured for the samples of the laboratory prepared mixtures using the procedure described under 2.5.3. The concentration of the intact drug was calculated from its corresponding regression equation.

Determination of NFL in pharmaceutical dosage form by the proposed methods

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 volume was completed to 100 mL using methanol, and then 10 milliliters of the filtrate were transferred into 100-ml volumetric flask and the volume was completed with methanol to obtain a concentration of 100 µg mL-1. The general procedures were followed.

RESULTS AND DISCUSSION

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

The degradation process under the previously mentioned conditions was followed using TLC and the compound was found to be stable under acidic and oxidative conditions but it is liable to degradation in alkaline condition giving one component which is confirmed with a previous study on stability of NFL[6].

This work is concerned with determination of

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NFL in presence of its alkaline degradate.

To detect the complete degradation of NFL, a TLC procedure was suggested. Different systems were tried, where complete separation of NFL from I was achieved using methanol-chloroform (20-80 by volume) as the mobile phase. The Rf values were 0.79 for NFL and 0.71 for I. 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 successful for separating NFL from I, except methanol-ethyl acetate-ammonia in the ratio (5-5-0.1 by volume) and methanol-chloroform in the ratio (2-8 by volume). 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. It was one component in case of alkaline degradate as indicated by the appearance of one spot of alkaline degradate after complete degradation and also confirmed by IR.

A suggested structure for alkaline degradate as shown in Figure 2[6].

As shown in Figure 2 the structure of the second degradate (b) has no UV-absorption as it is an aliphatic compound.

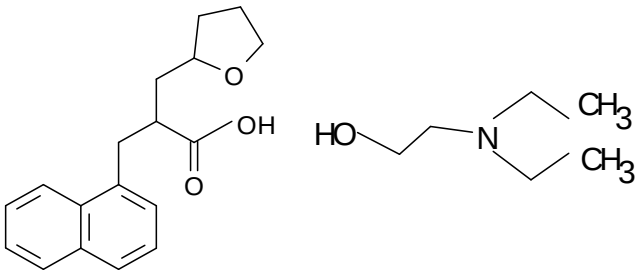

Figure 2 : Suggested structures for alkaline degradation products.

Upon scanning the absorption spectra of each of NFL and I, it was observed that NFL has a λmax at 272.6 and 282.6nm, at this wavelengths, I show complete overlapping as shown in Figure 3. Trials to use zero order absorption at 272.6 and 282.6nm for determination of NFL in presence of I was failed.

This work concerned with the determination of NFL in presence of its alkaline drgradate (I).

Applying first derivative ratio, the peak amplitudes at 249.2 nm was used for selective determination of NFL in presence of I in the range of 10-90 µg mL-1 as in Figure 4. Careful choice of the divisor and the working wavelength were of great importance, so different concentrations of I (10, 20, 30,….90) µg mL-1 were tried as divisors. It was found that the best one was 90 µg mL-1 of as they produce minimum noise and give better results in agreement with selectivity.

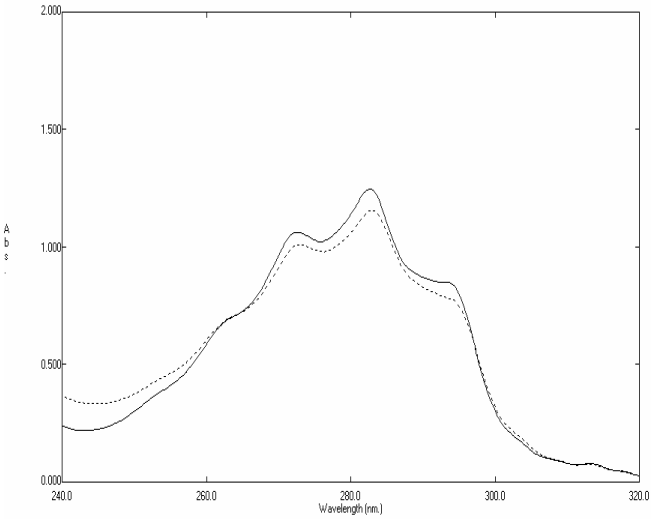

Figure 3 : Absorption spectra of a solution of NFL 90 µg mL^{-1} (——), its alkaline degradation product 90 µg mL^{-1} (……).

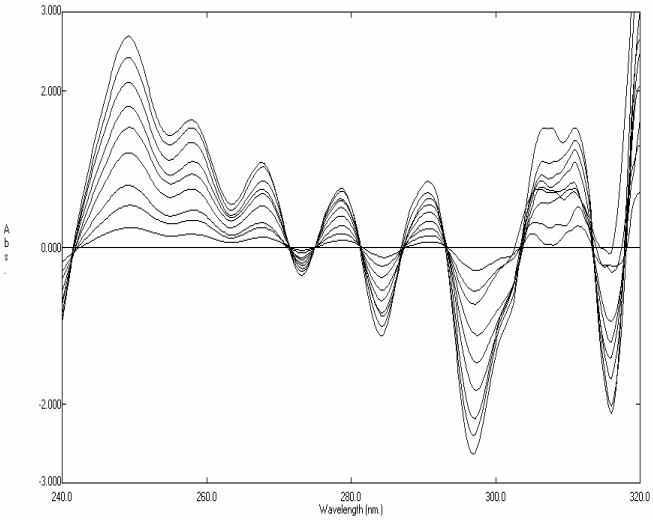

Figure 4 : First order of ratio spectra of NFL 10-90 using 90 µg.mL^{-1} of its alkaline degradation product as a divisor.

TLC-Densitometric technique allows selective determination of NFL in presence of I in range of 0.6-8 µg spot-1.

The regression equations were calculated and found to be:

A=0.0329 C - 0.0997 r = 0.9998 …1DD in presence of I at 249.2 nm

A=1881.9561 C + 3026.1941 r = 0.9994 … TLC-densitometry

Where, "A" is the peak amplitude in 1DD and peak area in densitometry, respectively. "C" is the concentration in µg mL-1 in 1DD and µg spot-1 in TLC-densitometry and "r" is the regression coefficient.

The mean percentage recoveries and standard deviations of the pure drug were calculated as shown in TABLE 1. The selectivity of the proposed method was assessed by the analysis of laboratory prepared mixtures containing different ratios of NFL and its degradate. The results shown in TABLE 2 contributed to the good performance of the methods with high selectivity to determine the studied drug in presence of up to 90% in presence of I in 1DD and to more than 90% in TLC-densitometry.

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 tech-

TABLE 1 : Determination of pure NFL by the proposed methods.

^{1}DD at 249.2nm In presence of I			TLC-Densitometric method		
Taken (µg mL^{-1})	Found (µg mL^{-1})	Recovery%	Taken (µg mL^{-1})	Found (µg mL^{-1})	Recovery%
10.00	10.03	100.30	0.60	0.59	98.33
20.00	19.96	99.80	2.00	2.06	103.00
30.00	29.83	99.43	4.00	4.05	101.25
40.00	39.74	99.35	6.00	6.08	101.33
50.00	50.29	100.58	8.00	7.92	99.00
60.00	60.95	101.58			
70.00	69.67	99.53			
80.00	80.44	100.55			
90.00	89.61	99.57			
Mean ± S.D		100.08 ± 0.739			100.58 ± 1.898

TABLE 2 : Results of laboratory prepared mixtures of NFL in presence of its alkaline degradation product by the proposed methods.

degradate %	^{1}DD at 249.2 nm	degradate %	TLC-Densitometry	degradate %
20%	102.14	25%	97.67	20%
40%	99.44	50%	97.00	40%
60%	99.41	75%	101.00	60%
80%	96.96	90%	95.00	80%
				90%
	99.49 ± 2.12		97.67 ± 2.494	

degradate %	^{1}DD at 249.2 nm	degradate %	TLC-Densitometry	degradate %
20%	102.14	25%	97.67	20%
40%	99.44	50%	97.00	40%
60%	99.41	75%	101.00	60%
80%	96.96	90%	95.00	80%
				90%
	99.49 ± 2.12		97.67 ± 2.494	

TABLE 3 : Determination of NFL in Praxilane® film coated tablets by the proposed methods 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 %
^{1}DD	101.22 ± 0.036		20	98.62
			40	101.33
			50	99.15
			Mean ± S.D	99.70 ±1.437
TLC-densitometry	101.93 ± 2.404		1.00	99.35
			2.00	100.20
			4.00	100.10
			Mean ± S.D	99.88 ± 0.465

***Average of three different determinations.**

TABLE 4 : Validation results of the proposed methods for the determination of NFL.

Parameters Linearity	^{1}DD	TLC-densitometry
Slope	0.0329	1881.9561
Intercept	-0.0997	3026.1941
Correlation coefficient	0.9998	0.9994
Range	10.00-90.00 µg mL^{-1}	0.60-8.00 µg/spot
Accuracy (Mean±S.D)	100.08 ± 0.739	100.58 ±1.898
Precision (RSD %)		
Repeatability[a]	0.423	1.442
Intermediate precision[b]	1.448	2.244

The intraday (n=3), average of three different concentrations repeated three time within the day.

The intermediate precision (n=3), average of three different concentration repeated three times in three days

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nique, as in TABLE 3, mean percentage recovery revealed that there was no interference from any excipients,that may be found in the pharmaceutical dosage forms.

Method validation was performed according to BP 2004[1] for all the proposed methods as 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 5.

TABLE 5 : Statistical analysis of the results of the proposed methods and the official method for determination of pure NFL.

method	[1]DD	Densitometry	Reported method*[1]
Mean ± S.D	100.08 ± 0.739	100.85 ± 1.898	100.28±1.119
n	9	5	6
Variance	0.546	3.602	1.253
Student's t test	0.148 (2.160)	0.336 (2.262)	-
F value	2.29 (3.69)	2.87 (5.19)	-

***Non-aqueous titration with 0.1 M perchloric acid, determining the end point potentiometrically.**

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