Stability-Indicating HPLC Method for Determination of Naftazone in Tablets. Application to Degradation Kinetics and Content Uniformity Testing

M.I. Walash, F. Belal, N. El-Enany, M. Eid*, and **R.N. El-Shaheny**

Department of Analytical Chemistry, Faculty of Pharmacy, University of Mansoura, 35516, Mansoura, Egypt

Abstract

A simple, sensitive, stability-indicating HPLC method was developed and validated for the quantitative determination of the vasoprotective drug, naftazone in presence of its degradation products. The analysis was carried out on a Nucleosil 100-5 phenyl column (250 mm × 4.6 mm, 5 µm) using a mobile phase consisting of methanol–0.02 M sodium dihydrogen phosphate mixture (60:40, v/v) of pH 6.0. The analyses were performed at ambient temperature with a flow rate of 1.0 mL/min and UV detection at 270 nm. The method showed good linearity over the concentration range of 0.1–10.0 µg/mL with a lower detection limit of 0.032 and quantification limit of 0.096 µg/mL. The suggested method was successfully applied for the analysis of naftazone in its commercial tablets. Moreover, it was utilized to investigate the kinetics of alkaline, acidic and oxidative degradation of the drug. The apparent first-order rate constants, half-life times, and activation energies of the degradation process were calculated. The pH-rate profile curve was derived. Furthermore, the proposed method was successfully applied to the content uniformity testing of naftazone tablets.

Introduction

Naftazone (NFZ, 1,2-naphthoquinone-2-semicarbazone, Figure 1), is a haemostatic, veno-active drug that is reported to increase venous tone and has a capillary stabilizing effect. It has been used in venous insufficiency of the lower limbs and diabetic retinopathy in oral doses of 30 mg daily. NFZ was formerly given by injection (1). NFZ is metabolized in humans by reduction and glucuronidation (2).

Naftazone is not yet the subject of a monograph in any pharmacopoeia. Reviewing the literature revealed that, only four methods have been reported for the determination of NFZ in raw material and/or human plasma, these methods include TLC/simultaneous reflectance-transmittance spectrophotometry (3), cathodic and adsorptive stripping voltammetry (4), and polarography (5,6). All these methods lack the stability-indicating nature, and none of them was applied for the determination of NFZ in formulations. As far as could be determined, the present study is the first report on a stability-indicating HPLC method for the determination of NFZ in tablets.

The parent drug stability test guideline Q1A (R2) issued by International Conference on Harmonization (ICH) (7) suggests that, stress studies should be carried out on a drug to establish its inherent stability characteristics. Accordingly, the aims of the present study were to establish the inherent stability of NFZ through stress studies under a variety of ICH recommended test conditions (7), also, it was necessary to develop the first stability-indicating assay method for its determination in its commercially available tablets. Furthermore, the proposed HPLC method was applied to study the degradation kinetics of NFZ under different degradation stress conditions.

Experimental

Apparatus

Separations were performed using a Merck Hitachi L-7100 chromatograph equipped with a Rheodyne injector valve with a 20 µL loop and a L-7400 UV detector (Darmstadt, Germany). Chromatograms were recorded on a Merck Hitachi D-7500 integrator. Mobile phases were degassed using Merck L-7612 solvent degasser. Hanna pH-Meter (Bucharest, Romania) was used for pH adjustments.

Camag UV-lamp (S/N 29000), dual wavelength (254/366), 2 × 8 W (Muttenz, Switzerland) was used in the photo-stability study.

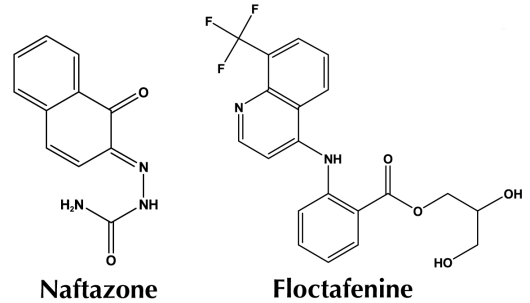

Figure 1. Chemical structures of naftazone and floctafenine.

* Author to whom correspondence should be addressed: Department of Analytical Chemistry, Faculty of Pharmacy, University of Mansoura, 35516, Mansoura, Egypt, email: manal_eid@yahoo.com

Materials

Naftazone pure sample (certified to have a potency of 99.9%), batch # 0301030075, was kindly provided by Alkan Pharma Co., 6th of October City, Egypt. Floctafenine, batch # 0607233200, was generously provided by Memphis Co. for Pharmaceutical and Chemical Industries, Cairo, Egypt. Mediaven tablets (labeled to contain 5 or 10 mg of NFZ/ tablet, batches # 012 or 016, respectively), products of Alkan Pharma Co., 6th of October City, Egypt, under license of Drossapharm-Switzerland, were purchased from local pharmacy. Methanol and acetonitrile (HPLC grade) were purchased from Sigma-Aldrich (Munich, Germany). Orthophosphoric acid (85%, w/v) was obtained from Riedel-deHäen (Seelze, Germany).

Hydrochloric acid (32%, w/v), hydrogen peroxide (30%, w/v), sodium hydroxide, boric acid and sodium dihydrogen phosphate were obtained from Adwic Co. (Cairo, Egypt). Glacial acetic acid was obtained from S.D. Fine-Chem. Ltd, Mumbai, India. Britton Robinson buffer was prepared by dissolving 5.0 g of boric acid in hot water, then adding 5.0 mL of glacial acetic acid and 5.0 mL of orthophosphoric acid. The mixture was then diluted to 1 L with distilled water. The required pH was then adjusted using 0.4 M solution of sodium hydroxide.

Chromatographic conditions

Column: Nucleosil 100-5 phenyl column (250 mm × 4.6 mm, 5 µm particle size), Macherey-Nagel, Bethlehem, PA. Mobile phase: A solution containing a mixture of methanol and 0.02 M sodium dihydrogen phosphate (60:40, v/v), the pH of the mixture was adjusted to pH 6.0 with 0.02 M orthophosphoric acid. The mobile phase was filtered through a 0.45 µm membrane filter (Millipore, Cork, Ireland). Flow rate: 1 mL/min. UV detector wavelength: 270 nm. Internal standard: floctafenine (standard solution containing 800 µg/mL of floctafenin was freshly prepared in methanol).

Preparation of standard solution

A standard solution containing 100 µg/mL of NFZ was prepared in methanol. Working solutions were prepared by appropriate dilution of the standard solution with the mobile phase. The standard solution was found to be stable for 1 week when kept in the refrigerator.

General recommended procedures

Procedure for calibration graph

Working solutions containing (0.1–10.0) µg/mL of NFZ were prepared by serial dilution of the standard solution together with an aliquot of the internal standard (final concentration of 80 µg/mL) with the mobile phase. 20 µL aliquots were injected (triplicate) and eluted with the mobile phase under the previously described chromatographic conditions. The average peak area ratios between NFZ and the internal standard were plotted versus the concentration of NFZ in µg/mL. Alternatively, the corresponding regression equation was derived.

Procedure for the analysis of tablets

Ten tablets were accurately weighed, finely pulverized and thoroughly mixed. An accurately weighed amount of pulverized tablets corresponding to 10.0 mg of NFZ declared active principle was transferred to 100 mL volumetric flask. Eighty milliliters of methanol was added and the mixture was sonicated in an ultrasonic bath for 30 min, the volume was completed with methanol and the solution was filtered. Aliquots of this solution together with the internal standard were successively diluted with the mobile phase and completed as under "Procedure for calibration graph". The nominal contents of the tablets were obtained either from the calibration graph or from the regression equation.

Procedure for content uniformity testing

The same procedure applied for the analysis of NFZ in tablets was followed using one tablet as a sample. Ten tablets were analyzed and the uniformity of their contents was tested by applying the official USP guidelines (8).

Procedure for alkaline degradation

Aliquots of the standard NFZ solution (containing 250 µg) were transferred into a series of small conical flasks; 5 mL aliquots of NaOH solutions (1.25×10^{-3}, 2.50×10^{-3}, 3.75×10^{-3}, or 5.00×10^{-3} M) were added. The solutions were heated in a thermostatically controlled water bath at different temperature settings (50°C, 60°C, 70°C, and 80°C) for different time intervals (5–50 min). At the specified time, the contents of each flask were cooled, neutralized to pH 7.0 and the solutions were then transferred into a series of 25 mL volumetric flasks. The volumes were completed with the mobile phase and triplicate 20 µL injections were made for each sample.

Procedure for acidic degradation

Aliquots of the standard NFZ solution (containing 250 µg) were transferred into a series of small conical flasks; 5 mL aliquots of HCl solution (0.125, 0.25, 0.375, or 0.50 M) were added. The solutions were heated in a boiling water bath for different time intervals (5–40 min). At the specified time, the contents of each flask were cooled, neutralized to pH 7.0, and the solutions were transferred into a series of 25 mL volumetric flasks. The volumes were completed with the mobile phase and triplicate 20 µL injections were made for each sample.

Procedure for oxidative degradation

Aliquots of the standard NFZ solution (containing 250 µg) were transferred into a series of small conical flasks; 5 mL aliquots of 15% H_2O_2 solution were added to each flask. The solutions were heated in a thermostatically controlled water bath at 80°C for different time intervals (15–60 min). At the specified time intervals, the contents of each flask were cooled, the solutions were transferred into a series of 25 mL volumetric flasks and the volume was completed with the mobile phase. Triplicate 20 µL injections were made for each sample.

Procedure for photolytic degradation

Aliquots of the standard NFZ solution (containing 250.0 µg) were transferred into two series of 25 mL volumetric flasks and 5 mL of either methanol, water, or methanol–water mixture (50:50, v/v) were added. The first series was exposed to UV-lamp at a wavelength of 254 nm at a distance of 15 cm placed in a wooden cabinet for 60 h. The second series of flasks was exposed

to sun light for 10 h. At the specified time, the volume was completed with the mobile phase. Triplicate 20 µL injections were performed for each sample.

The effect of pH

The effect of different pH values on the stability of NFZ was studied using Britton Robinson buffer of different pH values (2–11). Aliquots of methanolic NFZ solution (containing 250 µg) were transferred into a series of small conical flasks and diluted with 5 mL of buffer solutions at different pH values. Afterwards, these solutions were heated in a boiling water bath. Samples were withdrawn for the analysis at increasing time intervals. The solutions were cooled, neutralized to pH 7.0 then transferred into 25 mL volumetric flasks and completed to volume with the mobile phase. Triplicate 20 µL injections were made for each sample.

Results and Discussion

The proposed method permits the quantitation of NFZ in commercial tablets. Additionally; the content uniformity testing was performed. Figure 2A shows a typical chromatogram indicating good resolution of NFZ (t_R = 7.50 min) and floctafenine as internal standard (t_R = 11.65 min) under the described chromatographic conditions. The proposed HPLC method allows the separation of NFZ from all possible degradation products. Figures 2B, 2C, and 2D show good resolution of NFZ from each of its alkaline, acidic and oxidative degradation products, respectively. It was also possible to conduct stability study for the degradation kinetics of the drug.

Selection and optimization of chromatographic conditions

Well-defined symmetrical peaks were obtained upon measuring the response of eluent under the optimized conditions after thorough experimental trials. Two columns were used for performance investigations, including: Nucleosil 100-5 phenyl column (250 mm × 4.6 mm i.d., 5 µm particle size), Macherey-Nagel and Symmetry C_{18} column (250 mm × 4.6 mm i.d., 5 µm particle size), Waters, Dublin, Ireland.

Experimental studies revealed that, the first column was appropriate, giving symmetrical, well defined peaks and allowing good separation of NFZ from its degradation products.

The UV absorption spectrum of methanolic NFZ solution showed three maxima at 270, 320, and 440 nm, while those of its acidic and alkaline degradation products showed only one peak with maximum absorption at 270 nm. On the other hand, the oxidative degradation of NFZ didn't cause change in its absorption spectrum. Therefore, the UV detection was performed at 270 nm which allowed the detection of both the drug and all of its degradation products.

Several modifications in the mobile phase composition were performed in order to study the possibilities of changing the selectivity of the chromatographic system. These modifications included the change of the type and ratio of the organic modifier, the pH, the strength of phosphate buffer and the flow rate. The results achieved are summarized in Table I. Methanol and acetonitrile were investigated for selection of the proper organic modifier for the assay, but the latter did not produce well separated peaks. Methanol was the organic modifier of choice giving symmetrical, narrow and well-resolved peaks.

The effect of changing the ratio of organic modifier on the selectivity and retention times of the test solutes was investigated using mobile phases containing 50–80% (v/v) of methanol. Ratios more than 60% of methanol resulted in co-elution of the alkaline degradation product with the intact drug, and overlapping of the peak of the minor acidic degradation product with that of the major acidic degradation product. Meanwhile, mobile phases containing ratios less than 60% of methanol caused unacceptable long retention time of the drug and relatively broad peaks. Finally, mobile phase consisting of methanol–0.02 M sodium dihydrogen phosphate mixture in the ratio of 60:40,

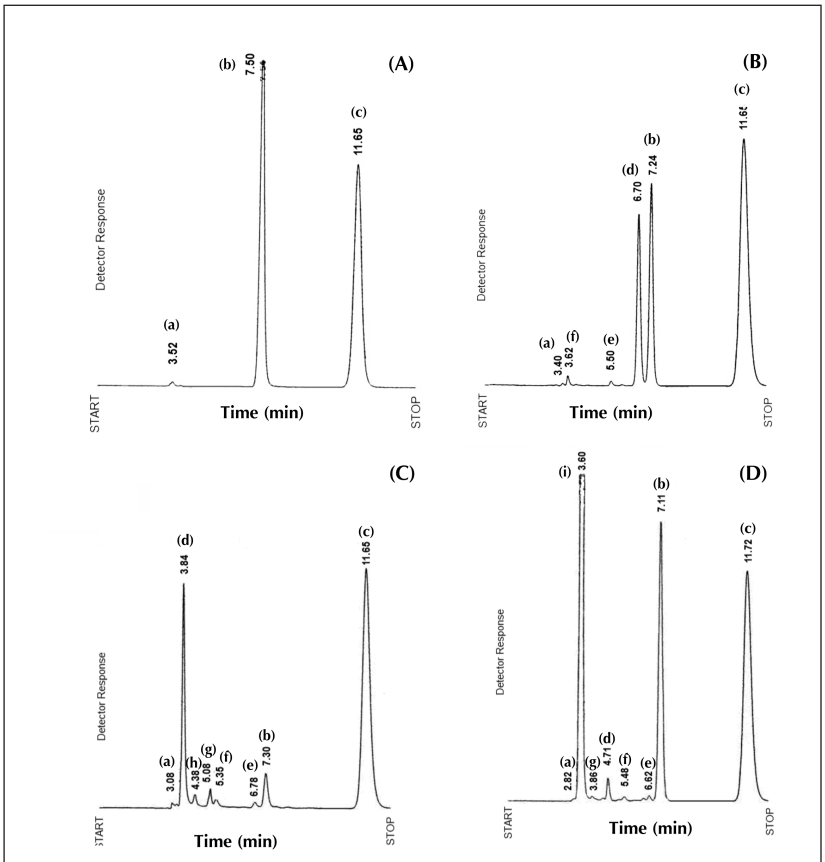

Figure 2. Representative chromatograms showing: (A) Standard NFZ (10 µg/mL) with internal standard (80 µg/mL), (B) Alkaline degradation of NFZ (10 µg/mL) with NaOH (5.00×10^{-3} M) at 70°C for 30 min, (C) Acidic degradation of NFZ (10 µg/mL) with HCl (0.5 M) at 100°C for 30 min, and (D) Oxidative degradation of NFZ (10 µg/mL) with H_2O_2 (15%) at 80°C for 30 min. Where; (a) Solvent front, (b) NFZ, (c) floctafenine (80 µg/mL), (d, e, f, g, h) degradation products, and (i) peroxide peak.

v/v (pH 6.0), was considered the optimal one as it gave a good compromise between retention times, resolution factor, number of theoretical plates, and peak shape.

To investigate the effect of the pH of the mobile phase on the selectivity and retention times of the test solutes, mobile phases of pH values ranging from 4.0 to 6.5 were attempted. With mobile phases of pH values higher or lower than pH 6.0, the peak of alkaline degradation product overlapped with that of the drug. Therefore, pH 6.0 was the most appropriate one permitting good separation of the drug from all of its degradation products.

The effect of changing the ionic strength of phosphate buffer on the selectivity and retention times of the test solutes was investigated using mobile phases containing concentrations of 0.002–0.030 M of phosphate buffer. Mobile phase containing phosphate buffer of ionic strengths greater than 0.02 M resulted in overlapping of the peak of alkaline degradation product with that of the drug, meanwhile, buffers of ionic strengths less than 0.01 M caused overlapping between the peaks of minor and major acidic degradation products of the drug. Table I shows that 0.02 M phosphate buffer (pH 6.0) was found to be the most suitable one giving good resolution and highest number of theoretical plates. The effect of flow rate on the separation of peaks of the studied drug and its degradation products was investigated and a flow rate of 1 mL/min was found to be the optimal one for good separation within a reasonable time (Table I). Flow rates greater than 1 mL/min caused overlapping of the peak of NFZ with that of its alkaline degradation product, while, flow rates lower than 1 mL/min caused long retention times.

Different drugs were investigated for the choice of a suitable internal standard. These drugs include; ziprasidone HCl, quetiapine fumarate, metformin, diclofenac sodium, aspirin, ibuprofen, chlorzoxazone, methocarbamol, guaifenesin, loratadine, felodipine, doxazosin, glafenine, and floctafenine. Floctafenine was found to be the best internal standard producing a well-resolved peak from the intact drug and each of its degradation products. Whereas, other investigated drugs resulted in overlapping peaks either with the drug or its degradation products or even eluted too late with long retention times.

Method validation

Linearity and range

Under the above described experimental conditions, a linear relationship was established by plotting peak area ratio for NFZ to the internal standard against NFZ concentrations. The concentration range was found to be 0.1–10.0 µg/mL. The high value of the correlation coefficient (r-value > 0.999) with small value of intercept indicate the good linearity of the calibration graph over the working concentration range. Statistical analysis of the data gave small values of the standard deviation of the residuals ($S_{y/x}$), of slope (S_b) and of intercept (S_a), and the % relative error (Table II) (9). Thus, indicating low scattering of the points around the calibration curve.

Limit of quantitation and limit of detection

The limit of quantitation (LOQ) and limit of detection (LOD) were determined according to ICH Q2 (R1) recommendations (10). The results are summarized in Table II.

Table II. Performance Data for the Proposed HPLC Method for Determination of Naftazone*

Parameter	Results
Concentration range (µg/mL)	0.1–10.0
Limit of detection (LOD) (µg/mL)	0.032
Limit of quantification (LOQ) (µg/mL)	0.096
Correlation coefficient (r)	0.9999
Slope	0.1402
Intercept	-4.09×10^{-3}
$S_{y/x}$	2.86×10^{-3}
S_a	1.35×10^{-3}
S_b	2.27×10^{-4}
% RSD	0.44
% Error	0.15

* $S_{y/x}$: Standard deviation of the residuals
S_b : Standard deviation of the slope
% Error = % RSD/√n
S_a: Standard deviation of the intercept

Table I. Optimization of the Chromatographic Conditions for Separation of Naftazone from its Degradation Products by the HPLC method

		No. of theoretical plates (N)				Mass distribution ration (Dm)				Resolution (Rs)			Relative retention (α)		
Parameter		NFZ*	a*	b*	C*	NFZ*	a*	b*	C*	NFZ/a*	NFZ/b*	NFZ/C*	NFZ/a*	NFZ/b*	NFZ/C*
Ratio of	60/40	7980	7340	12688	12285	1.11	0.11	0.85	0.51	11.67	1.26	6.68	1.96	1.08	1.51
organic	55/45	7649	8340	5591	5591	1.75	0.19	1.13	0.69	16.71	2.16	8.93	2.40	1.13	1.77
modifier†	50/50	7777	9267	10995	8571	2.99	0.34	1.18	0.87	16.78	5.58	11.2	2.85	1.31	2.00
Ionic	0.02	7880	7340	12688	12285	1.11	0.11	0.85	0.51	11.67	1.26	6.86	1.96	1.08	1.51
strength	0.01	7625	6943	6423	12817	1.20	0.07	0.95	0.55	11.26	1.94	6.50	2.11	1.0	1.54
of buffer	0.0075	7584	1735	6254	5532	1.22	0.10	0.98	0.54	9.70	1.36	6.21	2.10	2.10	1.56
	0.005	6555	2584	5069	1161	1.26	0.27	0.98	0.64	11.20	1.95	6.19	2.12	1.14	1.51
	0.002	4468	22268	5462	5864	1.37	0.31	1.08	0.69	13.27	1.53	5.20	2.26	1.13	1.48
Flow rate	0.8	4920	11520	9889	8953	1.15	0.13	0.82	0.45	11.15	1.33	5.57	1.98	1.10	1.55
(mL/min)	1.0	6980	7340	12688	12285	1.11	0.11	0.85	0.51	11.67	1.26	6.86	1.96	1.08	1.51

* NFZ: naftazone: a = Main acidic degradation product; b = Main alkaline degradation product; C = Main oxidative degradation product.
† (A/B) A = methanol and B = 0.02 M NaH_2PO_4.

LOQ and LOD were calculated according to the following equations:

$LOQ = 10S_a/b$
$LOD = 3.3S_a/b$

where, S_a is the standard deviation of the intercept of regression line and b is the slope of the regression line.

Accuracy

The accuracy of an analytical method is defined as the similarity of the results obtained by this method to the true values. To test the validity of the method it was applied to the determination of pure samples of NFZ over the concentration range of 0.1–10.0 µg/mL. The high percentages recoveries with average value of 100.09 and small value of S.D. (± 0.44) indicates the accuracy of the proposed method. The accuracy of the proposed HPLC method was also evaluated by studying the accuracy as percent relative error (% Error) and precision as percent relative standard deviation (% RSD), and the results are shown in Table II.

Precision

The intra-day precision was evaluated through replicate analysis of three concentrations of NFZ in pure form on three successive times. The inter-day precision was also evaluated through replicate analysis of three concentrations for a period of 3 successive days. The results of intraday and interday precision are summarized in Table III. The small values of RSD and % Error indicate high accuracy and precision of the proposed method, respectively.

Specificity

The specificity of the proposed HPLC method was proven by its ability to determine NFZ in its commercial tablets confirming that, there was no interference by common excipients and additives such as; anhydrous calcium phosphate, microcrystalline cellulose, pre-gelatinized starch, talk, and colloidal silicon dioxide. The placebo consisted of all these excipients omitting the active ingredient was prepared and 20 µL was injected under the described chromatographic conditions for the assay. As revealed from Figure 3A, these matrix components did not show any interfering peaks at the retention times of either the drug or any of its degradation products.

Specificity of the method was also confirmed by its ability to measure unequivocally the drug in the presence of all degradation products, as revealed by the results of the stability study (Figure 2B–2D).

Table III. Precision data for Determination of Naftazone Using the Proposed HPLC Method

Conc. (µg/mL)	% Recovery*	% RSD	% Error
Intra-day precision			
2.0	99.6 ± 1.5	1.5	0.9
4.0	100.7 ± 0.6	0.6	0.3
8.0	99.9 ± 1.0	1.0	0.6
Inter-day precision			
2.0	100.5 ± 0.7	0.7	0.4
4.0	100.8 ± 0.8	0.8	0.5
8.0	99.5 ± 0.9	0.9	0.5

* Each result is the average of three separate determinations.

Robustness

For the evaluation of the method robustness, one chromatographic parameter was changed while keeping all others unchanged. The studied variables included; pH of the mobile phase (6.0 ± 0.2), ratio of methanol in the mobile phase (60 ± 2%), and concentration of phosphate buffer (0.02 ± 0.005 M). These minor changes did not affect the separation and resolution of NFZ from its degradation products.

Solution stability and mobile phase stability

The stability of the stock solution was determined by quantitation of NFZ and comparison to freshly prepared standard solution. No significant change was observed in standard solution response, relative to freshly prepared standard. Similarly, the stability of the mobile phase was checked. The results obtained in both cases proved that the sample solution and mobile phase used during the assay were stable up to 7 and 3 days, respectively.

Applications

Dosage form analysis

The proposed method was successfully applied to the assay of NFZ in commercial tablets (Mediaven tablets; 5 and 10 mg). The average percent recoveries of different concentrations were based on the average of three replicate determinations (Table IV). Figures 3B and 3C show representative chromatograms for determination of NFZ in its tablets.

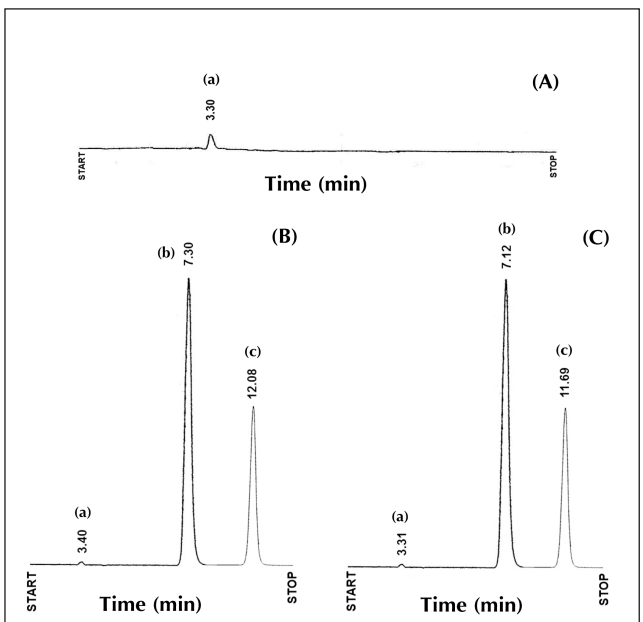

Figure 3. Representative chromatograms showing: (A) placebo and (B) NFZ (8 µg/mL) in Mediaven 5 mg tablets, and (C) NFZ (8 µg/mL) in Mediaven 10 mg tablets. (a) Solvent front, (b) NFZ, and (c) Floctafenine (80 µg/mL).

Content uniformity testing

Due to the high precision of the proposed method and its ability to rapidly estimate the concentration of the drug in a single tablet extract with sufficient accuracy, the method is ideally suited for content uniformity testing which is a time-consuming process when using conventional assay techniques. The steps of the test were adopted according to the USP (8) procedure. The acceptance value (AV) was calculated for each of the commercially available tablets and it was found to be smaller than the maximum allowed acceptance value (L1) stated by the USP (8). The results demonstrated excellent drug uniformity for both of Mediaven 5 and 10 mg tablets.

Degradation behavior of NFZ and study of its degradation kinetics

Liquid chromatographic studies on NFZ under different stress conditions suggested the following degradation behavior.

Degradation in alkaline conditions

The drug was found to be highly labile to alkaline hydrolysis. The degradation of NFZ in 0.1 M NaOH at 80°C was so fast that, whole of the drug was degraded within 5 min. Subsequently, studies were performed using weak strengths of NaOH solutions ranging from ($1.25 \times 10^{-3} - 5.00 \times 10^{-3}$) at 50°C, 60°C, 70°C, and 80°C in order to study the alkaline degradation kinetics of the drug. Degradation of NFZ under basic conditions gives a major alkaline degradation product with retention time of 6.70 min and two minor alkaline degradation products eluted at 3.62 and 5.50 min (Figure 2B). The alkaline degradation of NFZ was found to be temperature dependent (Figure 4A). Also, the alkaline degradation of NFZ was found to depend on the concentration of NaOH solution. The apparent first order rate constants and half-life times at each temperature and concentration were calculated (Table V). By plotting log K_{obs} values versus 1/T, Arrhenius plot was obtained (11). Arrhenius equation was found to be:

$$\text{Log } K = 10.80 - (4.152/T)$$

The activation energy was calculated and was found to be 19.0 K. Cal./ mol. This value is in accordance with the reported values of activation energy required for hydrolysis of amides (12).

Table IV. Application of the Proposed HPLC Method for Determination of Naftazone in Commercial Tablets*

Pharmaceutical preparation	Conc. taken (µg/mL)	% Found
Mediaven tablets	2.0	98.1
(10 mg NFZ/ tablet)	4.0	98.4
	8.0	100.7
$\bar{x} \pm$ S.D.		99.1 ± 1.4
Mediaven tablets	2.0	101.6
(5 mg NFZ/ tablet)	4.0	99.3
	8.0	100.2
$\bar{x} \pm$ S.D.		100.4 ± 1.1

*Each result is the average of three separate determinations.

Degradation in acidic conditions

The peak area of the drug gradually decreased with time upon heating at boiling temperature in HCl solution, forming a major degradation product eluted at t_R of 3.84 min and four minor degradation products eluted at 4.38, 5.08, 5.36, and 6.78 min (Figure 2C). The rate of acidic hydrolysis was slower as compared to that in alkali. The acidic degradation of NFZ was observed significantly at boiling temperature, while at lower temperatures the degradation was relatively low. The acidic degradation of the drug was dependent on the concentration of HCl (Figure 4B). The apparent first order degradation rate constants and the half-life times at each concentration were calculated (Table V).

Oxidative degradation

Considerable degradation of NFZ was observed under oxidative conditions when the drug was treated with 15% H_2O_2 solu-

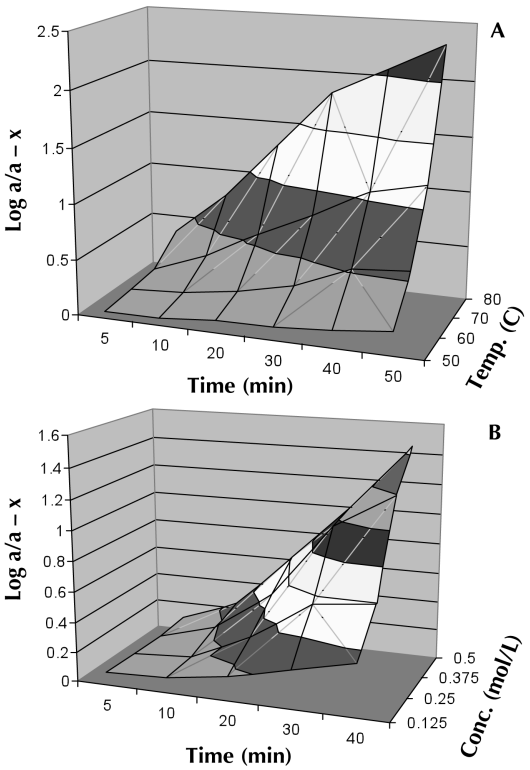

Figure 4. (A) Three-dimensional plot showing the effect of heating time with NaOH (5.00×10^{-3} M) on NFZ (10.0 µg/mL) at different heating temperatures. (B) Three-dimensional plot showing the effect of heating time (at 100°C) with different concentrations of HCl on NFZ (10.0 µg/mL).

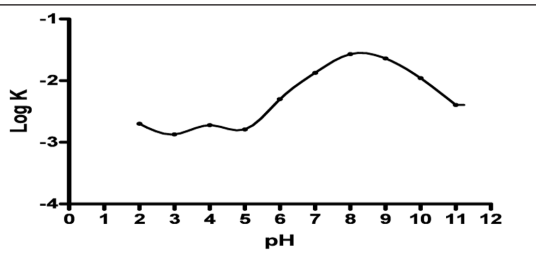

Figure 5. pH-Rate profile curve of NFZ using Britton Robinson buffer at 100°C.

tion and heated at 80°C. The oxidative degradation of NFZ was associated with the formation of a major degradation product eluted at 4.71 min and three minor degradation products eluted at 6.62, 5.48, and 3.86 min (Figure 2C). The oxidative degradation of NFZ was found to be time dependent. The apparent first order degradation rate constant and the half-life time were calculated and they were found to be 1.6×10^{-2} min^{-1} and 43.3 min, respectively.

Photolytic degradation

The drug was stable to UV-light and sun-light even after exposure for 60 h. Exposure of drug samples to acidic, alkaline and oxidative degradation at room temperature revealed that, the compound is stable under these conditions. A proposal for the degradation pathways of NFZ has been postulated and presented in Figure 6.

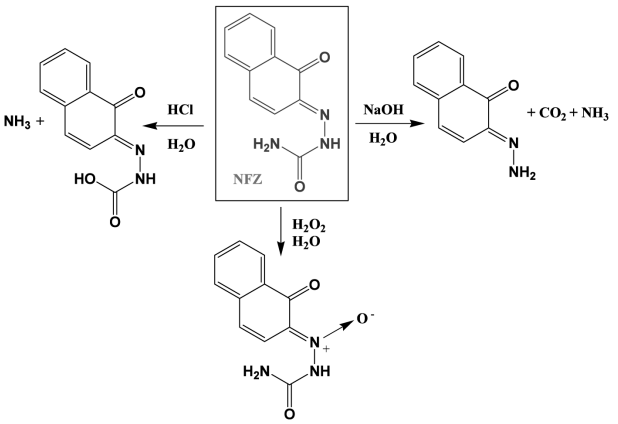

Figure 6. Proposal for the possible degradation pathways of NFZ under different degradation conditions.

Table V. Effect of Temperature and Concentration on the Kinetic Parameters of Degradation of Naftazone (10 µg/mL) Using Either NaOH or HCl

Medium	Temp. (°C)	K (min)	t½ (min)	Ea (K. cal. mol^{-1})
5.0×10^{-3} M NaOH	50	7.41×10^{-3}	94.0	26.82
	60	2.60×10^{-2}	27.0	15.31
	70	5.11×10^{-2}	14.0	16.06
	80	9.97×10^{-2}	7.0	**X** = 19.40

Medium	Conc. of NaOH or HCl (M)	K (min)	t½ (min)
NaOH (70°C)	1.25×10^{-3}	4.00×10^{-2}	17.3
	2.50×10^{-3}	4.31×10^{-2}	16.0
	3.75×10^{-3}	4.85×10^{-2}	14.3
	5.00×10^{-3}	5.11×10^{-2}	13.6
HCl (100°C)	0.125	1.80×10^{-2}	39.0
	0.250	3.80×10^{-2}	18.0
	0.375	7.80×10^{-2}	9.0
	0.50	8.90×10^{-2}	8.0

pH-rate profile study

The effect of different pH values on the degradation of NFZ was studied using Britton-Robinson buffer of pH values ranging from 2–11 at 100°C for different time intervals. The apparent first-order degradation rate constants were calculated at each pH and plotted versus the pH values resulting in pH-rate profile curve (Figure 5). The lowest degradation rate constant is at pH 3 at which the drug solution is most stable.

Conclusion

The present study represents the first report of a stability-indicating HPLC method for determination of NFZ in its commercially available tablets. The proposed method has the advantages of being simple, sensitive, accurate and precise, so that, it was applied to content uniformity testing of NFZ in its commercial tablets showing excellent drug uniformity. Moreover, the suggested method permits separation of the studied drug from its alkaline, acidic or oxidative degradation products within a reasonable time. Furthermore, the proposed method was successfully applied to study the kinetics of alkaline and acidic degradation processes of NFZ and to derive the pH-rate profile curve for the studied drug.

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