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Development of spectrofluorimetric stability indicating method for determination of naratriptan hydrochloride in pharmaceutical dosage form

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RESEARCH ARTICLE

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ABSTRACT

A stability indicating spectrofluorimetric method is developed for the determination of naratriptan hydrochloride in pharmaceutical formulation. The proposed method is based on investigation of the native fluorescence spectral behaviour of the drug in aqueous phosphate buffer (pH = 7.0±0.2).The fluorescence intensity is measured at 355.0 nm after excitation at 230.0 nm. The fluorescence-concentration plot is linear over the concentration range 8.0-80.0 ng/mL, with lower detection limit of 2.6 ng/mL and quantification limit of 7.6 ng/mL. The method is successfully applied to the analysis of the studied drug in its commercial tablet. Furthermore, the proposed method is applied in dissolution study of tablet; the results are in good agreement with those obtained with the reference method. The proposed method is approved to be a stability-indicating assay after exposure of the drug to different forced degradation conditions, such as acidic, alkaline and oxidative conditions, according to International Conference on Harmonization guidelines.

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1. Introduction

Naratriptan hydrochloride (NAR) (*N*-Methyl-3-(1-methyl-4-piperidinyl)-1*H*-indole-5-ethane sulfonamide monohydro chloride) is a selective serotonin (5-HT1) agonist (Figure 1) [1,2]. It is used for the acute treatment of the headache phase of migraine attacks. It should not be used for prophylaxis. It is given orally as the hydrochloride salt, and doses are expressed in terms of the base; NAR 1.11 mg is equivalent to about 1.00 mg of NAR base [1,3]. The recommended dose of NAR in the UK is 2.5 mg, and in the USA it is 1.0 or 2.5 mg [3].

Reviewing the literature has demonstrated some reported analytical techniques for the determination of NAR including voltammetry [4], spectrophotometry [5-8], densitometry [9], high-performance liquid chromatography (HPLC) with UV detection [2,10] and liquid chromatography-tandem mass spectrometry methods [11-14].

The key characteristic of fluorescence spectrometry is its high sensitivity. Fluorometry may achieve limits of detection several orders of magnitude lower than those of most other techniques. Because of the low detection limits, fluorescence is widely used for quantification of trace constituents in biological and environmental samples [15-17]. To the best of our knowledge, no spectrofluorimetric method has been published for determination of NAR, this motivated us to study and validate a simple sensitive and precise spectrofluorimetric method for determination of NAR in pure and dosage forms.

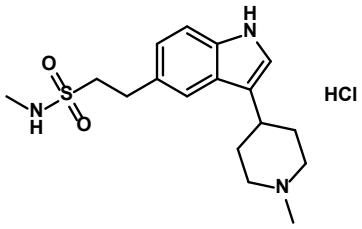

Figure 1. Chemical structure of naratriptan hydrochloride.

2. Experimental

2.1. Apparatus

All the fluorescence spectra were recorded using a JASCO FP-6200 Spectrofluorometer, equipped with 150 W Xenon lamp, grating excitation and emission monochromators, and a recorder. Slit widths for both monochromators were set at 10 nm and a 1 cm quartz cell was used for measurements.

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Table 1. Statistical analysis of the results of NAR in pure form by the proposed method, compared with comparison method [7].

Parameter	Proposed method			Comparison method [7]		
	Taken (ng/mL)	Found (ng/mL)	% Recovery	Taken (µg/mL)	Found (µg/mL)	% Recovery
	8	8.16	101.96	1	0.98	98.27
	10	10.16	101.60	3	2.99	99.99
	20	20.394	101.95	4	4.00	100.18
	40	39.33	98.32	5	4.98	99.63
	60	59.09	98.48	8	8.10	101.28
	80	80.91	101.13	10	9.93	99.26
Mean (X)	100.57			99.77		
± SD	1.71			1.00		
No of experiments	6			6		
Variance	2.92			1.00		
F-test	2.92 (7.14) *					
Students t-test	0.99 (2.23) *					

* Figures between parentheses are the tabulated F and t values, respectively, at p = 0.05 [20].

Spectra were evaluated using Spectra Manager FP-6200 Control Driver software [18], Version 1.54.03 [Build 1], JASCO Corporation.

2.2. Materials and reagents

2.2.1. Pure materials

Naratriptan.HCl (NAR), was purchased from SIGMA pharmaceutical industries (Osmopharm, Switzerland), its' percent purity was certified to be 99.6%.

2.2.2. Pharmaceutical preparations

Naredrix® Film coated tablet is manufactured by Organo for Pharmaceutical & Chemical industries, Elobour City industrial area, Egypt, were requested from commercial sources in the local market. Each tablet is labeled to contain 2.78 mg of NAR equivalent to Naratriptan base 2.5 mg (Batch Number1020774).

2.2.3. Reagents

All chemicals used were of analytical reagent grade and were used without further purification.Sodium dodecyl sulfate (SDS) (Rediel-De-Haen, Germany), 1.0 % (*w*:*v*) aqueous solution was prepared by dissolving 1.0 g SDS in distilled water, and then it was diluted to 100 mL with the same solvent. It is stable for seven days when left in the refrigerator. β-Cyclodextrin (β-CD) was obtained from Merck (Germany), 0.05 % (*w*:*v*) aqueous solution was prepared by dissolving 0.5 g β-CD in distilled water, and then it was diluted to 100 mL with the same solvent. Phosphate buffer (potassium phosphate monobasic) was obtained from (Sigma, Germany), 0.05 M aqueous solution pH = 7.0±0.2 was prepared according to the United States Pharmacopeia [2]. Hydrochloric acid HPLC grade 37% (Sigma, Germany), 2.0 M aqueous solution was prepared. Sodium hydroxide pellets (Winlab, Leicestershire, U.K.) 2.0 M aqueous solution was prepared. Methanol HPLC grade 99.9%, 2-propanol HPLC grade 99.9%, ethanol HPLC grade 99.8% and acetonitrile HPLC grade 99.9% were obtained from (Sigma, Germany)while sodium chloride was obtained from Winlab, Leicestershire, U.K.

2.3. Standard solutions

2.3.1. NAR stock solutions for calibration study

Stock solution (100.0 µg/mL) was prepared by accurately weighted 10 mg of NAR were dissolved in 100.0 mL of distilled water.

2.3.2. NAR working solution

A working solution (1000.0 ng/mL) was prepared by diluting accurately 1.0 mL of stock solution to 100.0 mL with distilled water.

2.4. Procedures

2.4.1. Procedure of calibration curve

Aliquots from NAR working solution equivalent to 80.0-800.0 ng NAR were carefully transferred into a series of 10 mL volumetric flasks. Then 2.0 mL 0.1 M phosphate buffer solution (pH = 7.0±0.2) were added to each flask. The volume was completed with distilled water; the contents of the flasks were mixed well. A blank experiment was performed simultaneously and the fluorescence intensity were Recorded at 355 nm after excitation at 230 nm and plotted against the final drug concentrations (ng/mL) to obtain the calibration curve. Alternatively, the corresponding regression equation was derived.

2.4.2. Procedure for tablets

Ten tablets (Naredrix®) were weighed and grinded well. An amount equivalent to one tablet (2.5 mg Naratriptan base) was accurately weighed, transferred to 50 mL volumetric flask and 6 mL 0.1 N NaOH was added followed by shaking for 30 min and sonication for 10 min. After neutralization with 6 mL 0.1 N HCl the volume was completed with phosphate buffer (pH = 7±0.2). The produced solution (50 µg/mL) was filtered then diluted to obtain aqueous stock solution (1.0 µg/mL). Aliquots from working solution covering the concentration range cited in Table 1 were transferred into a series of 10mL volumetric flasks, the "Procedure for calibration curve" was then performed. The tablet content was determined either from a previously plotted calibration curve or using correspondding regression equation.

2.4.3. Forced degradation

2.4.3.1. Alkaline and acidic degradation

Accurately weighted 10 mg NAR was transferred into a series of small conical flasks; 10 mL of 1.0 M NaOH or 1.0 M HCl were added. The flasks were heated in a water bath (80 °C) for different time intervals (1-10 hours). At the specified time intervals, the contents of each flask were cooled and neutralized to pH = 7.0 with 1.0 M HCl or 1.0 M NaOH, respectively. The solutions were then transferred into 100 mL volumetric flasks and diluted to volume with water. 1.0 mL of the resulting solutions was then transferred into 100 mL volumetric flasks and diluted to volume with water, 0.5 mL of resulting Solution were then transferred into 10 mL Volumetric flask and the "Procedure for calibration curve" was performed.

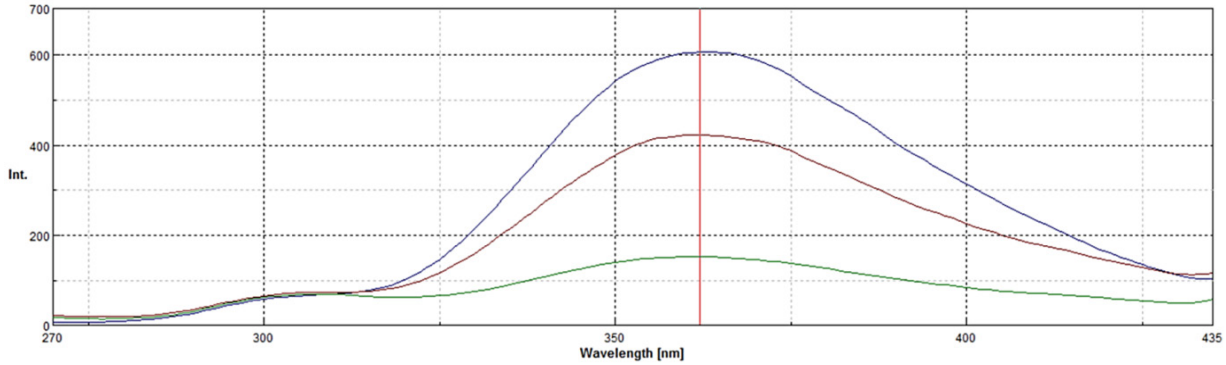

Figure 2. Emission spectra of NAR (10, 40 and 60 ng/mL) in aqueous phosphate buffer of pH = 7.0 after excitation at 230 nm.

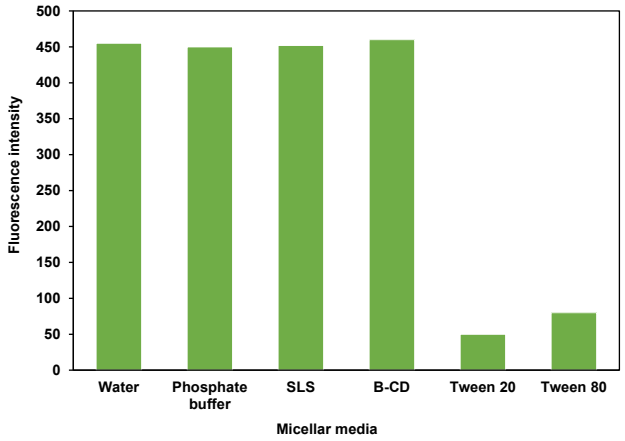

Figure 3. Effect of different micellar media on the fluorescence intensity of NAR (50 ng/mL).

2.4.3.2. Oxidative degradation

Accurately weighted 10 mg NAR was transferred into a series of 100mL volumetric flasks; 5 mL of 3% H_2O_2 were added and the volume was completed with water. 1.0 mL of this solution was transferred into 100 mL volumetric flask and diluted to volume with water, 0.5 mL of the resulting Solution were then transferred into 10 mL Volumetric flask and the "Procedure for calibration curve" was performed.

2.4.3.3. Photo-degradation

Accurately weighted 10 mg NAR was transferred into 100 mL volumetric flask and diluted to volume with distilled water. The volumetric flask was exposed to UV light in a wooden cabinet, for 6 hours. After time intervals from 1 to 6 hours 1.0 mL of this solution was transferred into 100 mL volumetric flask and diluted to volume with water, 0.5 mL of the resulting Solution were then transferred into 10 mL Volumetric flask and the "Procedure for calibration curve "was performed.

2.4.3.4. Thermal-hydrolysis

Accurately weighted 10 mg NAR was transferred into a series of small conical flasks and dissolved in 30 mL water. The flasks were heated at 90 °C for different time intervals (1-9 hours). At the specified time interval, the contents of each flask were cooled then transferred into 100 mL volumetric flask and diluted to volume with distilled water. 1.0 mL of this solution was transferred into 100 mL volumetric flask and diluted to volume with water, 0.5 mL of the resulting solution were then transferred into 10 mL volumetric flask and the "Procedure for calibration graph" was performed.

3. Results and discussion

NAR solution in aqueous phosphate buffer of pH = 7.0 is found to exhibit a strong native emission band with maximum fluorescence intensity (FI) at 355 nm after excitation at 230 nm (Figures 2). The fluorescence properties of NAR were carefully studied and optimized as following

3.1. Optimization of the experimental conditions

3.1.1. Effect of micellar media

The effect of different types of organized media (SDS, β-CD and Tween 80) on the fluorescence of NAR was investigated. Tween 80 caused severe decrease in the FI of the studied drug while the addition of SDS and β-CD caused no remarkable effect compared with aqueous solution (Figure 3).

3.1.2. Effect of diluting solvent

The effect of different diluting solvents (Methanol, ethanol, 2-propanol and acetonitrile) on the FI of NAR was studied. As shown in Figure 4, no significant difference is observed between the FI of the studied drug in the above-mentioned solvents in comparison with water, so water was selected as the optimum diluting solvent because it is safe and available low cost solvent, which add an economic advantage to the proposed method.

Table 2. Performance data of the proposed method for determination of NAR in pure form.

Parameter	Value
Concentration range (ng/mL)	8.0-80.0
Regression equation	FI = 8.983 × C + 72.23
Correlation coefficient (r)	0.9997
Standard deviation of the residuals ($S_{y/x}$)	6.83
Mean %Recovery	100.57
±SD	1.71
%RSD	1.7
% Er	1.61
LOQ (ng/mL)	7.6
LOD (ng/mL)	2.6

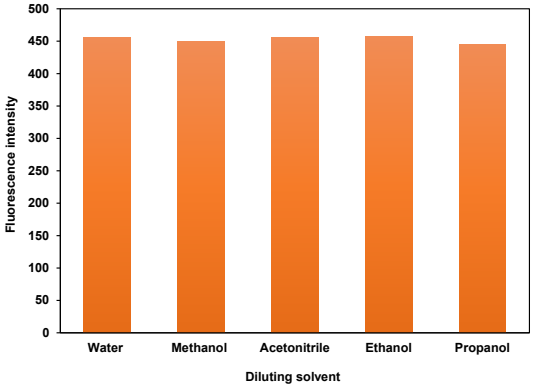

Figure 4. Effect of diluting solvent on the fluorescence intensity of NAR (50 ng/mL).

3.1.3. Effect of pH and type of buffer

The influence of pH on the fluorescence of NAR was studied using different types of buffers covering wide pH range, such as phosphate buffer, covering the pH range from 3.0 to 7.0 and 0.1 M *tris*-chloride buffer covering the pH range 7.0-11.0. It was found that maximum FI was achieved over the pH range 5.0-11.0; decreasing the pH value below pH = 5.0 caused gradual decreases in the FI of NAR (Figure 5). This effect may be attributed to the possible protonation of the secondary amino group in the indole ring of NAR which reduce conjugation and hence reduce fluorescence. Therefore, pH = 7.0 was chosen as the optimum pH value for measurement of FI of the studied drug.

3.1.4. Effect of time of fluorescence measurement

The effect of time of fluorescence measurement was studied by comparing the values of the FI obtained by measuring solution of the studied drug at different time intervals. Maximum fluorescence intensity was obtained when the studied drug solution was measured instantaneously after dilution and it remained constant for 2 hours.

3.2. Validation of the proposed method

The proposed method was tested for linearity, limit of quantitation (LOQ), limit of detection (LOD), accuracy, precision and robustness.

3.2.1. Linearity and range

After optimizing the measurement conditions, evaluation of linearity of the assay method was performed by analyzing eight concentrations of the studied drug (standard calibration plots). The calibration graph of NAR was constructed by plotting the FI of the studied drug at 355 nm against final concentration in ng/mL. The fluorescence-concentration plot was linear over the concentration range 8-80 ng/mL. Analysis of the data gave the following regression Equation (1),

$$FI = 8.983\ C + 72.23\ (r = 0.9997) \quad (1)$$

where FI is the fluorescence intensity, C is the concentration of the studied drug in ng/mL and *r* is the correlation coefficient. Calibration data of NAR in pure form by the proposed method are presented in Table 2. The good linearity of the calibration graph is indicated by the high value of the correlation coefficient (r) and small residual standard deviation ($S_{y/x}$) value, which measure the extent of deviation of the actual data points from the regression line.

The limit of quantitation was determined by establishing the lowest concentration that can be measured according to ICH Q2 (R1) recommendations [19], below which the calibration graph is non-linear. The limit of detection was determined by evaluating the lowest concentration of the analyte that can be readily detected.

The values of LOQ and LOD were calculated according to the Equations (2 and 3),

$$LOQ = 10\ \sigma / S \quad (2)$$

$$LOD = 3.3\ \sigma / S \quad (3)$$

where σ = The residual standard deviation of the regression line and S = Slope of the calibration curve. The results of LOQ and LOD are summarized in Table 2. The proposed method was evaluated by calculating accuracy as percent relative error and precision as percent relative standard deviation, the results are abridged in Table 2.

3.2.2. Accuracy and precision

The results of the proposed method were compared with those obtained using a comparison method [7].Statistical analysis [20] of the results using Student's t-test and Variance ratio F-test revealed no significant difference between the two methods regarding accuracy and precision, respectively as shown in Table 1.

Table 3. Accuracy and Precision data of the proposed method for the determination of NAR in pure and dosage form (Naridrex® Film coated tablet).

Parameter	Intra-day precision (Repeatability)			Inter-day precision (Intermediate precision)		
Concentration (ng/mL)	30	50	70	30	50	70
%Recovery, pure form	100.13	101.67	101.23	100.01	100.9	100.81
	99.02	104.01	99.33	102.35	102.23	102.02
	99.76	99.35	99.96	98.65	98.02	101.07
Mean (X)	99.64	101.68	100.17	100.34	100.38	101.3
± SD	0.57	2.33	0.97	1.87	2.15	0.64
%RSD	0.57	2.29	0.97	1.87	2.14	0.63
%Er	0.33	1.32	0.56	1.08	1.24	0.36
%Recovery, NAR tablets	94.96	94.69	94.57	96.07	96.02	95.53
	102.35	102.23	102.5	98.65	100.01	101.86
	99.76	101.35	99.33	99.76	99.13	99.96
Mean (X)	99.02	99.42	98.8	98.16	98.39	99.12
± SD	3.75	4.12	3.99	1.89	2.10	3.25
%RSD	3.79	4.15	4.04	1.93	2.13	3.28
%Er	2.19	2.39	2.33	1.11	1.23	1.89

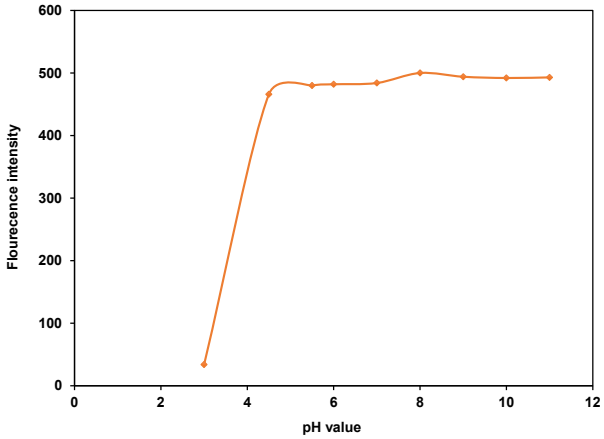

Figure 5. Effect of pH on the fluorescence intensity of NAR (50 ng/mL).

3.2.3. Repeatability

The repeatability (intra-day precision) was evaluated through replicate analysis of the studied drug in pure form and Naredrix tablet ® using three different concentrations (30, 50 and 70 ng/mL) and each concentration was measured three successive times. The results are summarized in Table 3.

3.2.4. Intermediate precision

The Intermediate Precision (inter-day precision) was evaluated through replicate analysis of the studied drug in pure form and in tablet on three successive days. The results are abridged in Table 3. The precision of the proposed method was fairly high, as indicated by the low values of SD and %RSD, respectively. Also, the inter-day and intra-day accuracy was proved by the low values of %Er.

3.2.5. Robustness of the method

The robustness of the proposed method was demonstrated by the constancy of the FI with minor changes in the experimental conditions, such as the pH = 7.0±0.5. The minor changes that may take place during the experimental operation did not affect the FI.

3.3. Pharmaceutical application

The proposed method was applied to the determination of NAR in its commercial dosage forms (Naridrex tablet ®). NAR is official drug in the USP and so is its tablet. The USP states that NAR tablets contain not less than 90.0 % and not more than 110.0% of the labeled amount. The dissolution analysis was carried out in USP suitable media, 0.1 N HCl [2].The results obtained by the proposed method were within the USP stated limit and they were compared with those obtained using the comparison method [7] as shown in Tables 4 and 5. Statistical analysis of the results obtained using Student's t-test and Variance ratio F-test revealed no significant difference between the performance of the two methods regarding accuracy and precision, respectively.

3.4. Results of stability indicating assay

NAR was found to be susceptible to acid degradation after heating at 80 °C with 1 M HCl for nine hours, where 18% of the drug was degraded as shown in Figure 6. In alkaline medium after heating the studied drug at 80 °C with 1.0 M NaOH for six hours, 17% of NAR was degraded. Increasing the heating time of the studied drug in alkaline medium up to nine hours caused dramatic increase in its degradation (55%) as presented in Figure 6, a predictable degradation bath way is shown in Figure 7. Oxidative degradation of NAR with hydrogen peroxide was also studied. It was found that the studied drug resists oxidative degradation after being treated with 3.0% H_2O_2 at room temperature. The effect of UV light on the stability of NAR was studied by exposing the aqueous drug solutions to the UV light. No considerable degradation was observed upon exposure of the drug to UV light up to six hours. The effect of heat on the stability of NAR was studied by heating the aqueous drug solutions at 90 °C for nine hours. No considerable degradation was observed under these conditions.

4. Conclusion

The spectrofluorimetric method proposed for the determination of NAR, has the advantages of simplicity, speed,

Table 4. Application of the proposed method for the analysis of NAR in dosage form (Naridrex® Film coated tablet).

Parameter	Proposed method		Comparison method [7]		
Conc. taken (ng/mL)	Conc. found (ng/mL)	% Recovery	Conc. taken (µg/mL)	Conc. found (µg/mL)	% Recovery
30.00	29.59	98.63	3.00	2.96	98.67
	30.70	102.34		3.01	100.33
	29.92	99.75		2.97	99.00
	29.70	99.00		3.01	100.17
	29.59	98.63		3.05	101.67
	30.81	102.71		3.09	103.00
50.00	49.74	99.49	5.00	5.06	101.20
	52.08	104.16		5.08	101.60
	51.19	102.38		4.95	99.00
	50.41	100.82		5.02	100.32
	49.49	98.98		5.10	102.00
	50.86	101.71		5.09	101.80
70.00	69.23	98.90	7.00	7.19	102.71
	71.90	102.72		6.95	99.29
	70.79	101.12		7.04	100.57
	71.01	101.44		7.17	102.37
	71.46	102.08		7.01	100.14
	70.12	100.17		6.91	98.71
Mean (X)	100.84			100.70	
Nominal content of Naratriptan in capsule (mg)	2.5			2.5	
± SD	1.70			1.41	
%RSD	1.69			1.40	
% ER	0.40			0.33	
No of experiments	18.00			18.00	
Variance	2.90			1.99	
F - test	1.458 (2.272) *				
Student's t-test	0.265 (2.032) *				

* Figures between parentheses are the tabulated F and t values, respectively, at p = 0.05 [20].

Table 5. Application of the proposed method for the analysis of NAR in dissolution suitable media USP at concentration 50 ng/mL.

Concentration found (ng/mL, 5 min)	% Recovery (5 min)	Concentration found (ng/mL, 10 min)	% Recovery (10 min)	Concentration found (ng/mL, 15 min)	% Recovery (15 min)
48.5	97	50.2	100.4	51.1	102.2
48.3	96.6	50.2	100.4	50.4	100.8
47.9	95.8	51.3	102.6	51.4	102.8
47.8	95.6	50.4	100.8	51.3	102.6
48.3	96.6	49.6	99.2	50.4	100.8
Parameter					
Mean (X)	96.32		100.68		100.84
± SD	0.59		1.20		0.97
%RSD	0.61		1.20		0.96
% ER	0.27		0.53		0.43

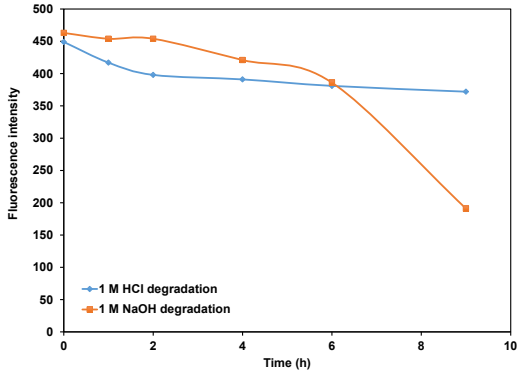

Figure 6. Plot of fluorescence intensity of NAR (50 ng/mL) versus heating time intervals with 1.0 M NaOH and 1.0 M HCl.

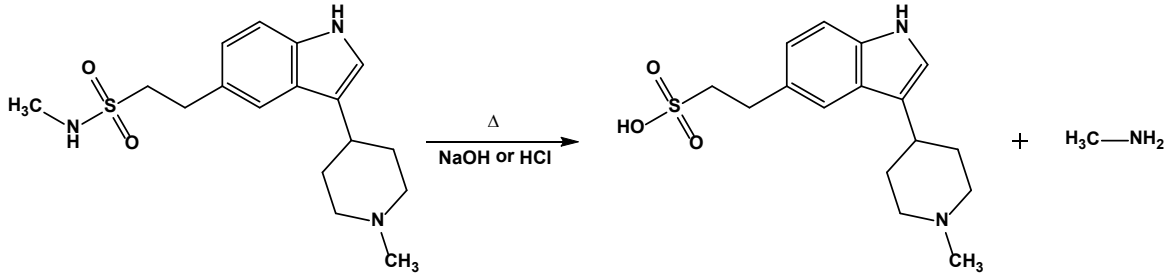

Figure 7. Predictable degradation bath way of NAR with 1 M NaOH and 1 M HCl.

accuracy, low detection limit and the use of inexpensive equipment, reagents and solvents. It can be considered as an attractive alternative to numerous other more complicated methods for determination of the studied drug. The proposed method could be successfully applied for the determination of NAR in pure form and in pharmaceutical preparations; the results are in good agreement with those obtained from comparison methods. Moreover, it has been adapted for stability studies of the drug as a rapid and simple alternative to the reported stability-indicating HPLC methods.

Disclosure statement

Conflict of interests: The authors declare that they have no conflict of interest.
Author contributions: All authors contributed equally to this work.
Ethical approval: All ethical guidelines have been adhered.
Sample availability: Samples of the compounds are available from the author.

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