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STABILITY STUDIES ON NICLOSAMIDE USING DERIVATIVE SPECTROSCOPIC AND CHROMATOGRAPHIC METHODS

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ABSTRACT

First derivative spectrophotometric, high-performance liquid chromatographic (HPLC) and thin-layer chromatographic (TLC) methods were developed for the study of the effect of the pH, alkaline and temperature on the degradation of niclosamide. The drug was found to undergo alkaline hydrolysis with insignificant temperature variation effects. The degradation process was found to resemble the metabolic hydrolytic cleavage of niclosamide to chlorosalicylic acid (fluroscent compound) and 2-chloro-4-nitroaniline (yellow colored product). The pH-rate profile of the alkaline pH- dependent hydrolysis of niclosamide was studied within the pH range 5-7 which indicate a first order dependency of K_{obs} on $[OH^-]$. In this study, a new stability-indicating thin-layer chromatographic method was also developed for the separation of the drug and its degradation products in addition to the photochemical stability study that shows the photosensitivity of niclosamide.

Keywords: Niclosamide; Stability-indicating; Derivative spectroscopic; Chromatography.

INTRODUCTION

Chemical and photostability studies of drugs and their formulations are an area of interest that developed into an important field of research. All Pharmacopoeias prescribe special packaging and storage conditions for hundreds of drugs and adjuvants.

Niclosamide (NA) is a potent anthelmintic drug (Fig. 1), which causes rapid disintegration of worm segments and solex.[1]

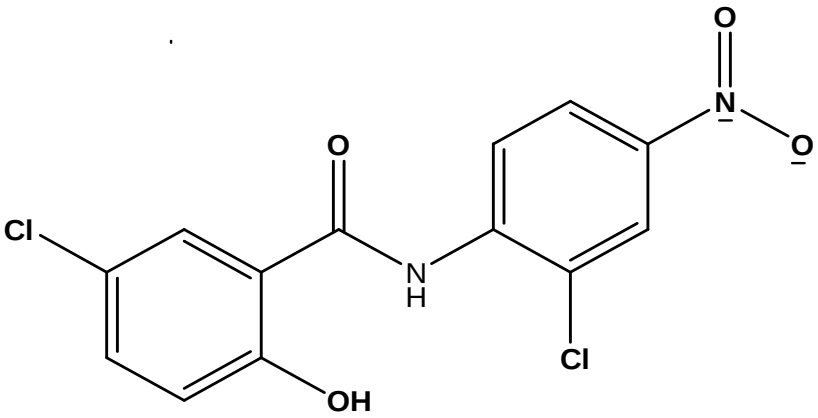


Figure 1: Chemical structure of Niclosamide

Various methods have been reported for the analysis of niclosamide in bulk and dosage forms .[2-5] None of these methods have been developed for the stability studies of the drug.

Therefore, the aim of the present work was to study the effects of pH, temperature, alkali and light on the stability of NA in bulk and dosage forms using the developed first derivative and HPLC and TLC methods.

EXPERIMENTAL

Instrumentation: UV spectrophotometric studies were carried out on Shimadzu UV-1800ENG240V, (Koyoto, Japan).

HPLC system consisted of a Shimadzu liquid chromatograph using Shimpack column VP-ODS (25 × 4.6 mm). The system was equipped with a UV-visible detector (SPD-20A prominence), P/N 7725i injector, 20 µL loop, DGU-20A3 prominence degasser and LC-20 AB pump.

Thin-layer chromatography (TLC) was conducted on a precoated silica gel sheets 60F254, 5 × 10 cm with 0.2 mm thickness.

Spectrophotometric method

The first derivative of NA zero-order spectrum was recorded over the range 320nm - 450 nm, which showed a peak at 351nm.

Chromatographic conditions

Chromatographic separation was achieved with the mobile phase methanol: water (70:30 v/v). The water was filtered through 0.45 µm membrane filter before being used. The mobile

phase was pumped through the column at a flow rate of 1 ml min^{-1}. The UV detector was set at wavelength 320 nm and the injected volume was 20 µl at room temperature.

The solvent system used in TLC was acetonitrile: methanol: ammonia: acetic acid (65:30:3.5:1.5). Visualization of the resolved spots was accomplished under UV light (254 nm and 365nm) Which was then followed by spraying with ferric chloride solution.

MATERIALS

A drug sample of NA (Niclosamide tablets, 500mg) was obtained from AlexiPharma, Egypt. The reference standard, certified to contain 99%, was obtained from Egypt. Disodium orthophosphate (BDH) Limited Poole, England; Sodium hydroxide (BDH), Pool, England; Acetonitrile (Lobachemie, India); Methanol Scharlau Chemie S.A., Spain; Acetone Scharlau Chemie S.A., Spain. All other chemicals used were of analytical grade reagents. Mcllvaine universal buffer (pH range 2.2- 8) was prepared. [6] Ferric chloride solution (5% w/v) was prepared in distilled water.

Sample solution

NA sample solution (2.5 mg/ml) was freshly prepared in acetone. After filtration, 1ml of the filtrate was further diluted with methanol to obtain 100µg/ml solution (solution A).

PROCEDURES

Effect of pH on the stability of NA

Aliquots of solution A (0.4 ml) were added to seven volumetric flasks (10 ml each) containing 1ml of the Mcllvaine universal buffer pH 2.2, 3, 4, 5, 6, 7 and 8 respectively.

Volumes were then completed to 10 ml with methanol and the solutions were mixed gently. The kinetics of the degradation of the drug was then monitored by the developed first derivative spectrophotometric method.

Effect of alkali on the stability of CS solution

Aliquots of solution A (0.4 ml) were transferred to five stoppered glass tubes. The volumes were then completed to 10ml with sodium hydroxide (0.1M). The first derivative spectrum for the solution in the first tube was recorded. The rest four solutions were heated in a boiling water bath for 10, 20, 30 and 40 min respectively. The solutions were then cooled. The effect of the alkaline on the degradation of niclosamide was monitored by the first derivative

spectrophotometry at (320 - 450 nm) and the HPLC method. The same procedure was repeated using 0.5 M and 2 M sodium hydroxide solution.

Effect of temperature on the stability of Niclosamide: Aliquots of solution A (0.4 ml) were added to eight round bottom flasks containing 1ml phosphate buffer pH 6. Volumes were completed to 10 ml with suitable amounts of methanol. Each four solutions were then heated under reflux at two appropriate temperatures (70º and 80º C) at time intervals ranging between 10 - 40 minutes. The reaction was then quenched by cooling and the kinetics of the degradation of the drug was then monitored using the first derivative spectrophotometric method.

Effect of light (photodegradation): Methanolic NA solution (10µg/ml) in a quartz cell was placed in a wooden cabinet 3 cm apart from the light source. The solution was then irradiated at 254nm at different time intervals of 0,30,60,90,120 minutes. The kinetic parameters of the photodegradation reaction were monitored by the derivative spectrophotometric method.

Another methanolic niclosamide solution (10µg/ml) in a glass bottle was irradiated at time intervals ranged between 1 - 6hrs. The photodegradation reaction was monitored by the first derivative spectrophotometry.

The effect of sunlight was also studied by exposing 10µg / ml solution of niclosamide) in glass bottle to direct sunlight. The photodegradation was monitored by the first derivative spectrophotometry at time intervals ranged between 1 - 6 hrs.

Thin-Layer Chromatography

Stock solution of NA (1mg/ml) was freshly prepared in methanol. 1ml of this solution was transferred to stoppered glass tube and 1ml of 0.1M sodium hydroxide was added. TLC plate containing in addition to the hydrolysed NA spot of unhydrolysed NA was developed to monitor the drug hydrolysis using the mobile phase acetonitrile: methanol: ammonia: acetic acid (65:30:3.5:1.5).The plate was then visualized under the UV-light (254 nm and 365nm) and then sprayed with ferric chloride solution.

RESULTS AND DISCUSSION

Knowledge of the chemical stability of a drug is of great concern for the selection of the suitable storage conditions against the effects of light, temperature, humidity..etc and for anticipation of drugs interaction with each other or with the excipients. [7,8]

Many drugs are derivatives of carboxylic acid or contain functional groups based on this moiety such as esters and amides. Accordingly, their susceptibility to nucleophilic attacks can result in a various chemical reactions that can result in their degradation. One of the most important types of drugs instability is the chemical hydrolysis. Amides are generally more stable to hydrolysis than esters due to their low reactivity. In general, the rate of hydroxyl ion-catalyzed hydrolysis of amides is greater than that of proton-catalyzed hydrolysis. [7]

NA contains an unusual amide group which show high susceptibility to chemical degradation through hydrolysis due to the electrons withdrawing effect that exerted by the nitro-substituted aromatic ring and the two chloro substituents (Fig 2). The first derivative spectrophotometry and HPLC methods were applied as stability-indicating methods to study the effect of different conditions (pH, alkali and light) on the stability of NA.

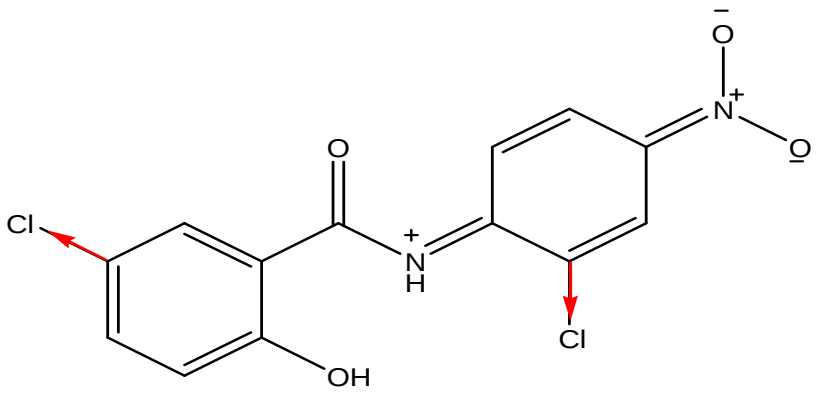


Figure 2: Electron withdrawing effect on the amides that result in its high susceptibility to nucleophilic hydrolysis

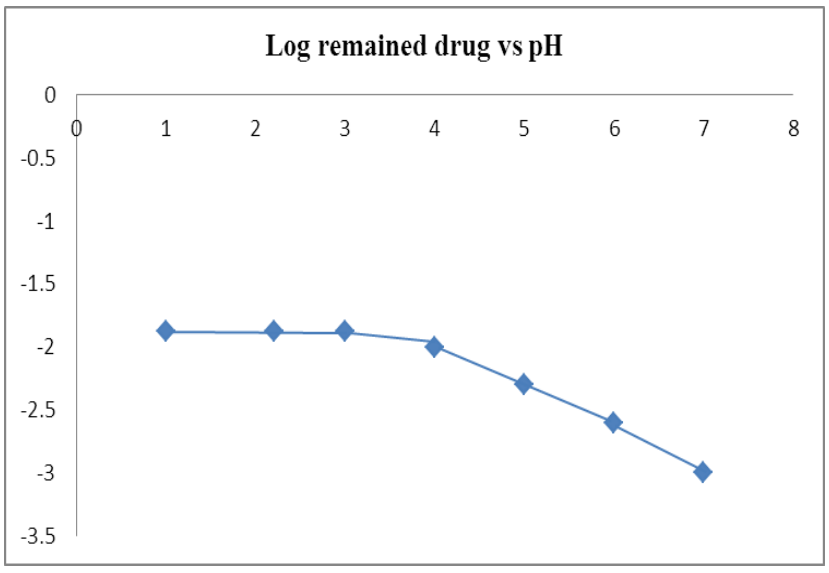


Figure 3: Effect of pH on the degradation rate of NA

Effect of pH on the stability of NA

The degradation of NA was monitored by the first derivative spectrophotometric method over the pH range 2.2 - 8 at room temperature (Fig. 3). The results obtained showed that the rate of the hydrolysis increases as pH increase. At pHs more than 4, a fast hydrolysis took place even at room temperature. This finding suggested that the stable formulation of the drug in liquid form should be at pHs less than 4 and stored at low temperature.

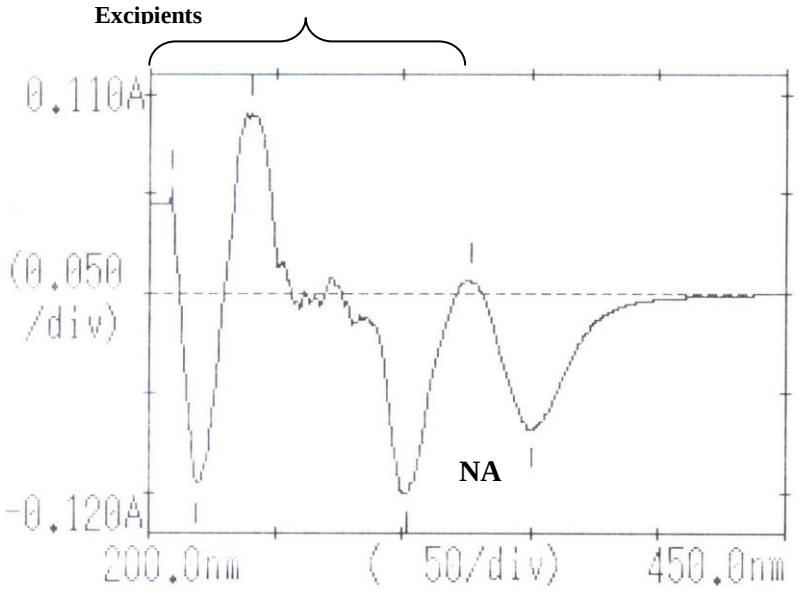


Figure 4. First derivative spectrum of NA (6µg/mL, 351nm)

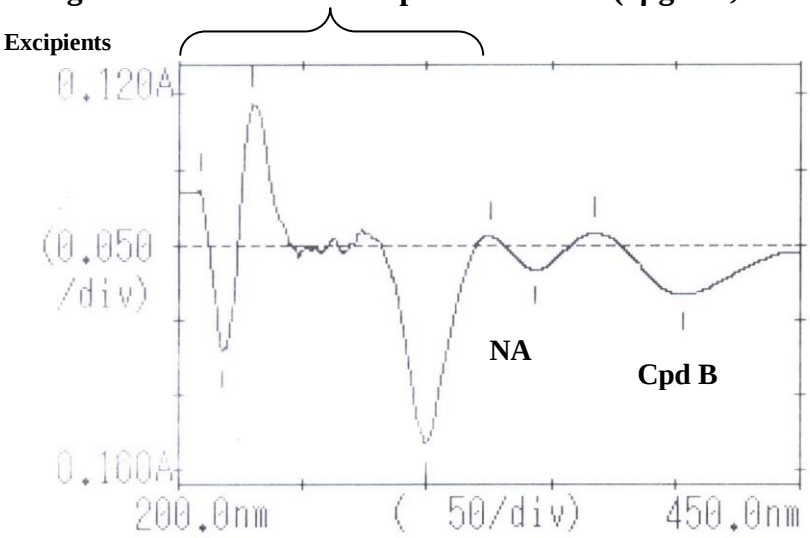


Figure 5: First derivative spectrum of degraded NA with 0.1M NaOH

UV spectral changes in NA solution treated with alkali

The first derivative spectrum of NA solution (Fig. 4) showed a major absorption peak at 351nm. Treatment of this solution with 0.1M sodium hydroxide resulted in the gradual

disappearance of this peak and the appearance of another major one at 409nm (Fig. 5) which supposed to be an alkali-induced degradation product of NA

Effect of alkali on stability of NA

The effect of different sodium hydroxide concentrations on niclosamide solution was studied using the developed first derivative spectrophotometric and the HPLC methods. The first derivative spectral changes of NA solution treated with 0.1M sodium hydroxide showed a decrease in niclosamide peak at its λ_{max} 351nm and the formation of a yellow colored degradation product with λ_{max} 409 nm (Fig. 5).The peak of the drug at 351nm was observed to disappear gradually upon heating the alkaline solution at time intervals 10, 20, and 30 minutes.

The plot of log % drug remaining vs time for three time intervals reflected a first order reaction (Fig. 6). The results obtained were summarized in Table 1.

Table 1. Effect of alkali on NA degradation at 100 ° C at time intervals 10-30 minutes

Time (min)	Absorbance	% Remained	Log %
0	0.0050	100	2
10	0.0030	60	1.778
20	0.0019	38.5	1.585
30	0.0010	20	1.301

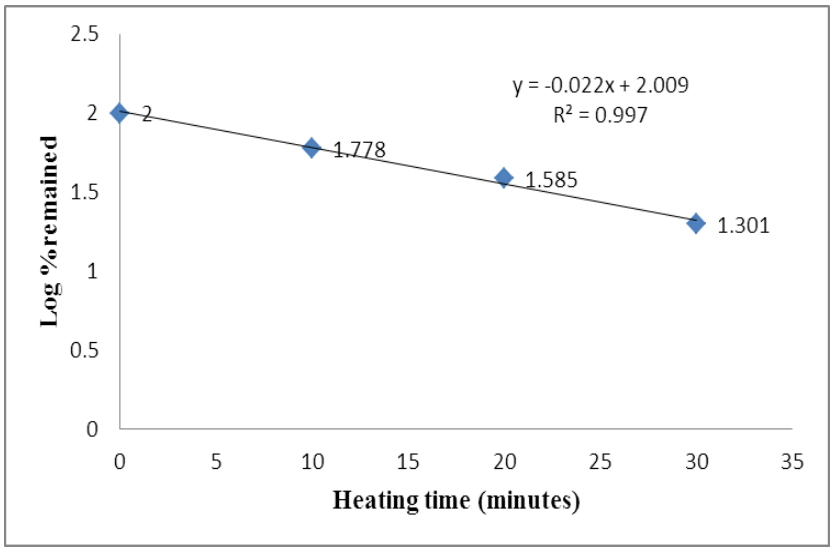


Figure 6: Effect of sodium hydroxide (0.1M) in the degradation of NA

The degradation rate constant (K_{obs}) was calculated by the linear regression analysis of the plot in which the slope equals to $-K_{obs}/ 2.303$.The degradation half life ($t_{½}$) and shelf-life (t_{90}) were then calculated as $0.693/K_{obs}$ and $0.1054/K_{obs}$ respectively (Table 2).

Table 2. Slope, K_{obs}, $t_{½}$ and t_{90} values for the degradation of NA at 100 °C in 0.1 M NaOH

Slope	K_{obs} (min^{-1})	$t_{½}$ (min.)	t_{90} (min.)
-0.023	0.053	13.08	1.98

Using the HPLC method, NA eluted at retention time of 3.5 minutes (Fig. 7). Fig. 8 reflects a typical chromatogram for NA solution treated with sodium hydroxide. The peak of the parent drug reflected a decrease in its concentration and the subsequent appearance of peaks for the degradation products at retention time of 2.256 minute (major/more polar degradant), 3.433 and 3.719 minutes (about same polarities).

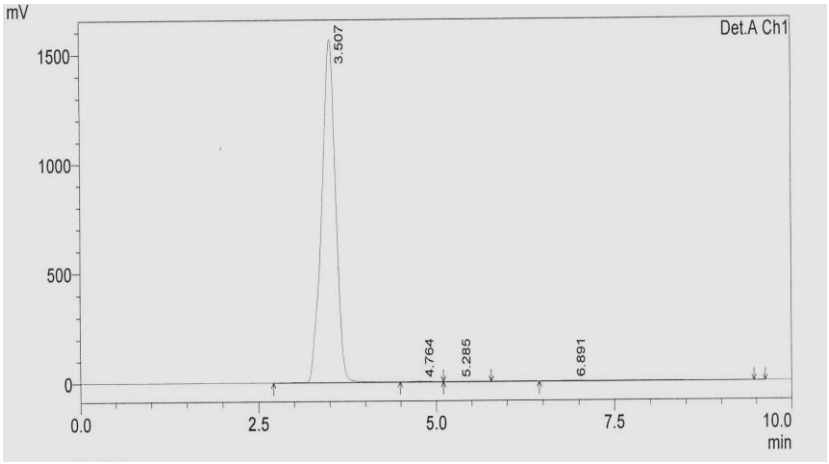


Figure 7: Typical chromatogram of the intact drug

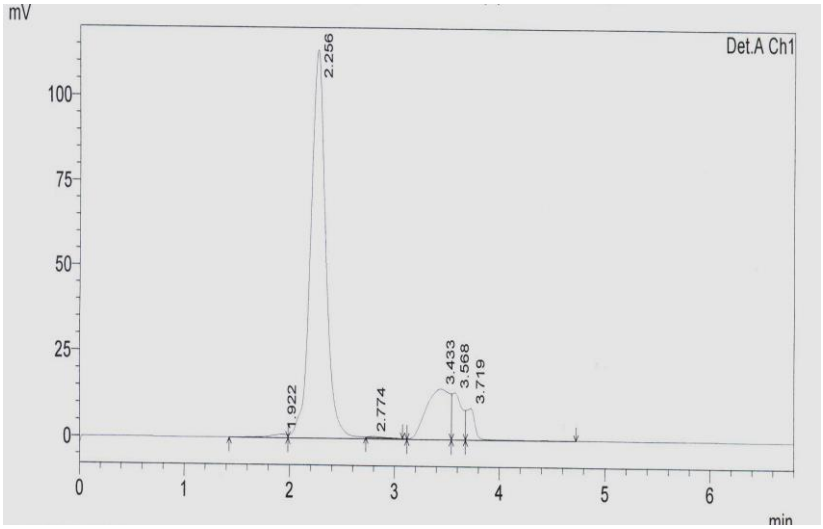
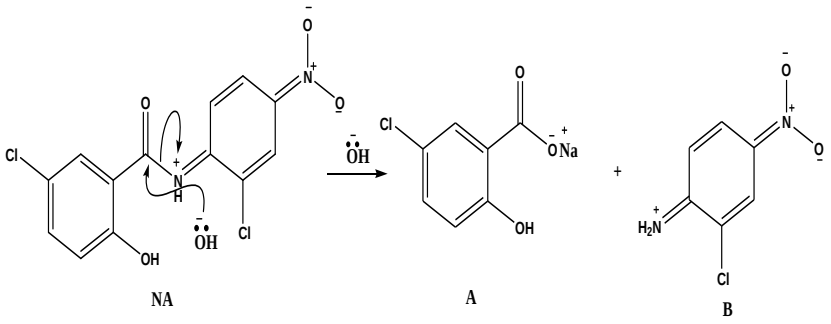


Figure 8: Typical chromatogram for NA degradation using 0.1M NaOH

High concentration of sodium hydroxide (1M) resulted in the immediate disappearance of the niclosamide peak (351nm) and the appearance of the degradation product peak at 409nm. The

immediate degradation in 1M NaOH can be more understood with referring to the $t_{½}$ and t_{90} values of the drug in 0.1M NaOH (13.08 and 1.98 minutes respectively).

The shift in wavelength maxima indicates the formation of a product with highly extended conjugation which leads to the formation of the yellow colored degraded product, scheme 1. The colored product is expected to be the (B) product which contains the strong electron withdrawing groups (NO_2 and Cl) and the electron donating NH_2 group. Product (A) is expected to be the flourescent UV absorbing compound.



Scheme 1: Proposed pathway for niclosamide degradation

Effect of temperature on NA stability

The effect of the temperature on the rate of NA degradation was studied at three different temperatures (70°, 80° and 100°) at pH 6 which is the pH at which NA was found to be unstable (Figure 3). Fast degradation was observed with no significant difference of the temperatures variation on the degradation rate of NA. Therefore, Arrhenius plot was not possible to be constructed and hence, the energy of activation could not be calculated.

Effect of light (photodegradation)

The official monograph [9] directs that NA should be protected from light. Based on this direction, a study that may provide information about the photostability of NA was conducted under defined conditions utilizing the developed stability-indicating spectrophotometric method.

First derivative spectrum of irradiated NA solution with sunlight for 2 hours showed a well separated peak at 264nm from the parent peak (351nm). The peak of the photodegradation product was found to increase gradually depending on the exposure time (Fig. 9).

Direct sunlight was found to cause photodecomposition of NA in glass bottles in a manner similar to that caused by the UV irradiation at 254nm in glass bottles or quartz cell.

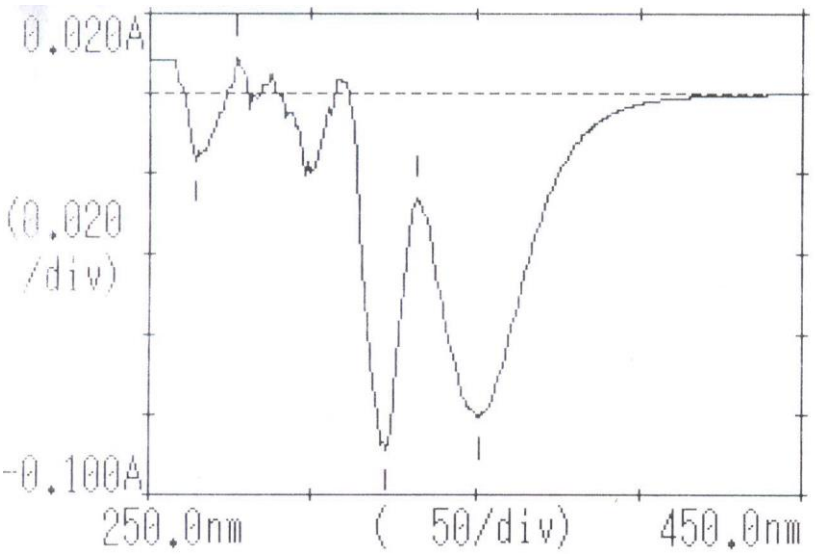


Figure 9: First derivative spectrum of irradiated NA solution with sunlight (2hours)

Thin-Layer chromatography

In order to obtain a suitable solvent for the separation of NA and its degradation product, mixtures of solvents with different polarity and composition ratios were investigated. The most appropriate mobile phase consisted of acetonitrile: methanol: ammonia: acetic acid (65:30:3.5:1.5) with a running time of about 15 minutes for 7 cm distance. This solvent successfully separated the drug and its degradation products. The R_f values were 0.87, 0.69 and 0.43 respectively.

The degraded product (R_f value 0.43) was found to be fluorescent when detected under UV light (wave length 365nm). It had been reported [10] that the chloro-derivatives of salicylic acid have fluorescent properties. Therefore, the degraded product (R_f value 0.43) is probably compound A (5-chlorosalicylic acid).

Ferric chloride solution was also used as conformational test. It was used to spray the plate after developing depending on the presence of phenolic groups in the intact drug and the cholorsalicylate degradation product. This indicated that the observed two red spots of the R_f values 0.87 and 0.43 are for NA and compound (A) respectively.

The developed stability-indicating thin-layer method can be utilized to separate and identify the NA degradation products.

CONCLUSION

The results of stability study on NA under alkaline conditions showed that it degrades quickly via a hydrolysis process which appeared to be [OH-] dependant. The degradation products were successfully separated using the developed stability-indicating derivative spectrophotometric, HPLC and TLC methods. NA was found to degrade in 0.1M NaOH to form a yellow colored product at 409nm and suggested to be the product (B).

NA was found to be stable at pHs ranging between 1-4 and starts to degrade at pH > 4 even at room temperature.

NA was observed to be a photosensitive drug and degrade upon exposure to light (sunlight and UV light). Therefore, It should be protected from light as directed by the B.P.

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