Accepted Manuscript


# Kinetic Study and Mechanism of Niclosamide Degradation

Hala A. Zaazaa, Maha M. Abdelrahman, Nouruddin W. Ali, Maimana A. Magdy, M. Abdelkawy

PII: S1386-1425(14)00623-4
DOI: http://dx.doi.org/10.1016/j.saa.2014.04.050
Reference: SAA 12014

To appear in: *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*

Received Date: 2 February 2014
Revised Date: 1 April 2014
Accepted Date: 7 April 2014

Please cite this article as: H.A. Zaazaa, M.M. Abdelrahman, N.W. Ali, M.A. Magdy, M. Abdelkawy, Kinetic Study and Mechanism of Niclosamide Degradation, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* (2014), doi: http://dx.doi.org/10.1016/j.saa.2014.04.050

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

# Kinetic Study and Mechanism of Niclosamide Degradation

**Hala A. Zaazaa[a] , Maha M. Abdelrahman[b] , Nouruddin W. Ali[b], Maimana A. Magdy[b*] , M. Abdelkawy[a]**

[a] Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr-El-Aini 11562, Cairo, Egypt.

[b] Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Beni-Suef University, Al-Shaheed Ahmed Hegazy, 26111, Beni-Suef, Egypt.

## Abstract

A spectrophotometric kinetic study of Niclosamide alkaline degradation as a function of drug concentration, alkaline concentration and temperature has been established utilizing double divisor-ratio spectra spectrophotometric method. The developed method allowed determination of Niclosamide in presence of its alkaline degradation products; namely; 2-chloro-4-nitro aniline (DEG I) and 5-chloro salicylic acid (DEG II) with characterization of its degradation mechanism. It was found that degradation kinetic of Niclosamide followed pseudo-first order under the established experimental conditions with a degradation rate constant ($k$) of 0.0829 mol/h and half life ($t_{1/2}$) of 8.35 h. The overall degradation rate constant as a function of the temperature under the given conditions obeyed Arrehenius equation where the activation energy was calculated to be 3.41 kcal/mol.

**Keywords**: Niclosamide; Double divisor-ratio spectra spectrophotometry; Degradation; Kinetic.

***corresponding author E-mail: maimanamagdy@yahoo.com**
**Telephone: 0201006773868**
**Fax : 0020822317950**

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

## 1. Introduction

Niclosamide (**Fig. 1**), chemically recognized as 5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide [1]. Niclosamide is orally administered anthelmintic drug which is highly effective against cestodes and threadworm. It acts by inhibiting the oxidative phosphorylation in mitochondria and interfering with anaerobic generation of ATP by the tape-worm, as a result tape-worm get partially digested in the intestine [2-4].

After extensive literature survey, few analytical methods have been found for determination of Niclosamide including determination of Niclosamide with Thiabendazole by ΔA spectrophotometric method [5] or in combination with Drotaverine hydrochloride by ΔA, second derivative ($\Delta D^2$) and third derivative ($\Delta D^3$) differential ultraviolet spectrophotometric methods[6], or alone using P-benzoquinone [7]. Also spectrofluorimetric [8], HPLC [9-11], GC [12] and vlotammetric [13-14] methods were developed for its determination.

Neither stability investigation of Niclosamide nor possible degradation mechanism has been reported. Niclosamide was subjected to alkaline hydrolysis and the obtained degradation products were isolated and characterized for their structures. They are reported as major impurities of Niclosamide namely; 2-chloro-4-nitro aniline (DEG I) and 5-chloro salicylic acid (DEG II) in British pharmacopoia [2] and the European pharmacopoia [15]. Additional results from genotoxic studies in rodents and humans suggest that the drug is absorbed from the gastrointestinal tract, and mutagenic metabolites 5-chlorosalicylic acid and 2-chloro-4-nitroaniline are excreted as the main metabolites [16].

The aim of the recommended work is to liberate double divisor-ratio spectra spectrophotometric method for selective determination of Niclosamide in presence of its degradation products. The proposed method was directed to reveal the degradation kinetic and mechanism of Niclosamide under the specified conditions.

## 2. Experimental

### 2.1. Instruments

- A double beam UV-visible spectrophotometer (SHIMADZU, Japan) model UV-1601 PC with quartz cell of 1 cm pathlength, connected to IBM compatible computer. The software was UVPC personal spectroscopy

ACCEPTED MANUSCRIPT

software version 3.7.The spectral band width was 2 nm and wavelength-scanning speed 2800 nm/min.

- UV lamp with short wavelength 254 nm (USA).

**2.2. Materials**

**(a) Pure standard**

- Niclosamide was kindly supplied from Alexandria Company for pharmaceuticals and chemical industries, Alexandria, Egypt. Its purity was found to be 100.00 % according to the reported method [5].

**(b) Pharmaceutical formulations**

- Yomesan® tablets (Batch No. 1182010) labeled to contain 500 mg of Niclosamide , manufactured by Alexandria Company for pharmaceuticals and chemical industries, Alexandria, Egypt.
- Niclosan® tablets (Batch No. 103021) labeled to contain 500 mg of Niclosamide , manufactured by Misr Company for pharmacutical Industry. S.A.E, Egypt.

**(c) Chemicals and reagents**

All reagents and chemicals used throughout this work were of analytical grade and were used without further purification.

- Methanol and triethylamine of HPLC grade (CHROMASOLV®, Sigma-Aldrich Chemie Gmbh, Germany).
- Sodium hydroxide (0.5 N and 1N aqueous solution), HCl (1N aqueous solution), benzene, ethylacetate (El-Nasr Pharmaceutical Chemicals Co., Abu-Zabaal, Cairo, Egypt).

**(d) Degradation of Niclosamide**

0.5 gm of Niclosamide powder was transferred into 50 mL glass stoppered flask, dissolved in 10 mL methanol and completed to the mark with 1N NaOH solution. The flask was left for 6 h under reflux at 80 ˚C and complete degradation was followed via TLC using benzene–ethylacetate- methanol- triethylamine (9:1:1:0.1, by volume) as a developing system. After complete degradation, the solution was filtered, the obtained precipitate was washed with water where the collected washing was identified as first

ACCEPTED MANUSCRIPT

degradation product DEG I (2-Chloro-4-nitroaniline). Then the filtrate was adjusted to pH=1.5 using 1N HCl solution, where the second degradation product DEG II (5-chlorosalicylic acid) precipitated, filtered and then washed with double distilled water. The obtained degradation products were identified by IR and mass spectrometry.

**2.3. Standard solutions**

**(a) Stock standard solutions of Niclosamide and its alkaline degradation products (1 mg/mL)**

0.1 gm of Niclosamide, DEG I and DEG II were accurately weighed into three separate 100-mL volumetric flasks, 50 mL of methanol was added to each flask, shaken to dissolve then the volume was completed to the mark with methanol.

**(b) Working standard solutions of of Niclosamide and its alkaline degradation products (100 µg/mL)**

10 mL each of Niclosamide, DEG I and DEG II were accurately transferred from their stock standard solutions into three separate 100-mL volumetric flasks, then the volume was completed to the mark with methanol.

**(c) Kinetic investigation**

Into two separate 100 mL volumetric flasks, standard solution of Niclosamide ($3.06x10^{-3}$ and $1.53x10^{-3}$ mol/ L) were prepared by dissolving in 10 mL methanol and then the volume was completed to the mark with 1N NaOH and 0.5N NaOH.

**2.4. Laboratory prepared mixtures**

Mixtures containing different ratios of Niclosamide, DEG I and DEG II were prepared using their respective working solutions in methanol. Different aliquots equivalent to 100-300 µg of Niclosamide were transferred into a series of 10 mL volumetric flasks, then different aliquots of DEG I and DEG II were added in different ratios according to their molecular weights.

ACCEPTED MANUSCRIPT

## 3. Procedure

### 3.1. Spectral characteristics of Niclosamide and its degradation products

Into 10-mL volumetric flasks transfer accurately and separately, aliquots equivalent to 180 µg of Niclosamide and 60 µg of each of DEG I and DEG II from their working standard solutions (100 µg/mL). Complete the volume with methanol and record the absorption spectrum over the range of 200-500 nm.

### 3.2. Double divisor-ratio spectra spectrophotometric method

Different aliquots equivalent to 40-300 µg of Niclosamide were accurately transferred into a series of 10 mL volumetric flasks and the volume was completed to the mark with methanol. Divide the absorption spectrum by the spectra of mixture of equal concentrations of both of DEG I and DEG II (10 µg/mL of each). The $^{1}D$ spectra of Niclosamide was obtained using $\Delta\lambda$ = 5 and scaling factor =10, then the peak amplitude was measured at 243 nm. The calibration curve was constructed relating the peak amplitudes against the corresponding drug concentrations and the regression equation was calculated.

### 3.3. Application to the pharmaceutical formulations

Ten tablets of each Yomesan® and Niclosan® were powdered and mixed well. An accurately weighed portion of the powdered tablets equivalent to 500 mg of Niclosamide of each formulation was transferred separately into 100-mL volumetric flasks. 75 mL methanol was added and then sonicated for 30 minutes, filtered then completed to the volume with methanol. The solution was diluted to obtain 100 µg/ mL working solution using methanol. The double divisor-ratio spectra spectrophotometric method was followed and the concentration of Niclosamide was calculated from its corresponding regression equation.

### 3.4. Kinetic study

#### 3.4.1. Effect of Niclosamide concentration

Two sets of standard solutions of Niclosamide ($3.06x10^{-3}$ and $1.53x10^{-3}$ mol/L) were separately prepared in 100 mL glass stoppered flasks after dissolving in 10 mL methanol then the volume was completed to the mark with 1N NaOH solution, inserted as rapidly as possible into a thermostatic water bath set at 60 °C, 0.3 mL from the prepared solutions were quantitatively transferred into 10-mL volumetric

ACCEPTED MANUSCRIPT

flasks for 6 h at 0,1,2,-----,6 h time interval. The flasks were completed to volume with methanol. The remaining concentration of intact Niclosamide was determined by applying the previously described double divisor-ratio spectra spectrophotometric method at 243 nm using its corresponding regression equation. The logarithm of the remaining Niclosamide concentration was plotted versus time in hours from which the kinetic order of alkaline degradation process was determined, the degradation rate constant ($k$) and the half life ($t_{1/2}$) were calculated.

### 3.4.2. Effect of sodium hydroxide concentration on the reaction rate

Two sets of Niclosamide standard solutions ($3.06x10^{-3}$ mol/L) were separately prepared in 100 mL glass stoppered flasks after dissolving in 10 mL methanol then the volume was completed to the mark with 0.5N and 1N NaOH solutions. Then the prepared solutions were inserted into a thermostatic water bath set at 60 °C. The procedure was followed as mentioned under **3.4.1**.

### 3.4.3. Effect of temperature on the reaction rate

Two sets of Niclosamide standard solutions ($3.06x10^{-3}$ mol/L) were separately prepared in 100 mL glass stoppered flasks after dissolving in 10 mL methanol then the volume was completed to the mark with 1N NaOH solutions. Then the prepared solutions were inserted into a thermostatic water bath set at 60 °C and 80 °C. The procedure was followed as mentioned under **3.4.1**. Arrehenius plots were used to determine the activation energy ($E_a$), half life of Niclosamide ($t_{1/2}$) and frequency factor (A).

## 4. Results and Discussion

Generally, some amide containing pharmaceutical compounds are liable to hydrolysis in aqueous solution. Upon refluxing Niclosamide with 1 N NaOH for 6 h at 80 ˚C, hydrolysis of the amide group occurs and two degradation products were obtained. The first degradation product is 2-chloro-4-nitro aniline (DEG I) filtered and washed with water, then the filtrate is adjusted to pH =1.5 with 1 N HCl solution where the other degradation product, 5-chloro salicylic acid (DEG II) precipitated. The two degradation products were identified by IR and mass spectrometry where IR spectrum of Niclosamide showed a characteristic bands at 3576.34 $cm^{-1}$ (peak 1) ,

ACCEPTED MANUSCRIPT

3088.44 $cm^{-1}$ (peak 2) and 1679.69 $cm^{-1}$ (peak 3) corresponding to -OH , -NH , and C=O groups respectively, while absence of peak 1 and peak 3 in the IR spectrum of the first degradation product (DEG I) and appearance of a forked characteristic bands at 3483.78 $cm^{-1}$ (peak 4) and 3371.92 $cm^{-1}$ (peak 5) corresponding to $-NH_2$ group **Fig.2**. Also the IR spectrum of the second degradation product (DEG II) showed a characteristic bands at 3226.33 $cm^{-1}$ (peak 6) and 1671.98 $cm^{-1}$ (peak 7) corresponding to -OH and C=O groups of the carboxylic acid and a characteristic bands at 3081.69 $cm^{-1}$ (peak 8) corresponding to the phenolic -OH **Fig. 2**.

The electron impact of Niclosamide showed mass ion peak at 327 m/z corresponding to the molecular weight of the intact drug while the mass ion peak of DEG I was at 173 m/z and its base peak at 80 m/z corresponding to the main fragment C5H6N and the mass ion peak of DEG II was at 173 m/z and its base peak at 154 m/z corresponding to the main fragment C7O2HCl as shown in Fig. 3. Therefore we can conclude that the alkaline hydrolysis of Niclosamide may be proceeds as shown in **Fig. 1**. Pure standards of 2-chloro-4-nitro aniline (DEG I) and 5-chloro salicylic acid (DEG II) were obtained from Sigma Aldrich [17] and it was compared with the isolated degradation products with TLC and melting point which confirm the results or IR and mass spectroscopy.

In accordance with the fact that Niclosamide is susceptible to hydrolysis in alkaline solution; development of a stability indicating assay method was critical and information about degradation kinetics under different experimental conditions was elaborated. According to the method based on the principle of kinetic developed by *Garrett and Carper* [18], the *k* value (reaction rate constant) for the degradation of the drug in alkaline solution at different elevated temperature was obtained from the linear expression in equation Log C= $\log C_0$ - *kt* /2.303 [19], where the slope of the line is $-k/2.303$. The log of the rates of degradation was then plotted against the reciprocals of the absolute temperature (Arrehenius plot) [20].

### 4.1. Double divisor- ratio spectra spctrophotometric method

Double divisor spectrophotometry [21-23], has proved usefulness in the determination of medicinal substances by eliminating specific interference from degradation products, co-formulated drugs and also the non-specific irrelevant absorption from the formulation matrix. The zero order absorption spectra of Niclosamide and its degradation products show serious overlap that direct determination of Niclosamide is

ACCEPTED MANUSCRIPT

not possible **Fig.4.** The problem of the overlapped spectra of Niclosamide and its degradation products was solved using the double divisor-ratio spectra spectrophotometric method.

The absorption spectra of Niclosamide, DEG I and DEG II solutions were divided by the spectrum of a solution containing DEG I and DEG II (10 µg/mL of each). Then $^{1}D$ spectra were obtained using scaling factor=10 and $\Delta\lambda$=5, where Niclosamide could be determined at 243 nm without any interefernce from DEG I and DEG II. The first derivative of the ratio spectra of pure Niclosamide and its ternary mixture with its degradation products containing the same concentration of Niclosamide as in its pure spectrum would be coincided in the spectral region corresponding to a maximum point of the wavelength as shown in **Fig. 5.**

Linear correlations were obtained between peak amplitudes at 243 nm for $^{1}D$ spectra of Niclosamide in the concentration range 4–30 µg/mL from which the regression equations were calculated and found to be:-

P.A= 0.0369 C + 0.0217 r= 0.9997

Where P.A is the peak amplitude of Niclosamide, C is the concentration in µg/mL and r is the correlation coefficient.

Results described in **Table 1** showed that this method is selective, valid and applicable for the determination of Niclosamide in presence of its degradation products in different laboratory prepared mixtures with mean recovery of 100.74 ±1.528. Method validation was performed according to USP [24] guidelines, **Table 2** shows results of accuracy, repeatability and intermediate precision, which shows good linear relationship for the suggested method as revealed by the correlation coefficient. **Table 3** shows statistical comparison of the results obtained by the proposed method and those obtained by the reported spectrophotometric method [5]. The calculated *t*- and F-values are less than the theoretical ones indicating that there is no significant difference between them with respect to accuracy and precision. The usefulness of the proposed method for assay of Niclosamide in its pharmaceutical formulations was studied by analysis their tablet formulation (Yomesan® and Niclosan® tablets). Furthermore, the validity of the proposed method was assessed by applying the standard addition technique, which showed accurate results and there was no interference from tablet excipients as shown in **Table 3.**

ACCEPTED MANUSCRIPT

### 4.2 Kinetic investigation

No kinetic spectrophotometric study has been reported in the literature for the assay of Niclosamide. The kinetic methods possess some specific advantages like; simplicity and no need of some experimental steps such as filtration, extraction which are avoided prior to absorbance measurements. The kinetic methods also are highly selective methods since they involve the measurement of the absorbance as a function of the reaction time [25-27]. This work describes a simple and sensitive kinetic spectrophotometric method for the determination of Niclosamide. The double divisor-ratio spectra spectrohotometric method was used to determine the order of the alkaline degradation rate of the reaction by following the decrease in concentration of Niclosamide within 6 h at 1 h time interval. Alkaline catalyzed hydrolysis is a bimolecular reaction but one of the reactants is water which is present in large excess so the change in concentration is negligible. Such reactions where one of the reactants is present in large excess are considered to follow pseudo-first order kinetics and all the equation describing first order can be applied for it.

For a given initial concentration of Niclosamide at a constant temperature and solution normality, by plotting the change of Niclosamide concentration versus time and a linear relationship between ln ($C_t/C_0$) and time shows that the degradation of Niclosamide followed a pseudo-first order reaction kinetics. The pseudo first order rate constant ($k$) was calculated as follows [20]:

$\ln C_t/C_0 = -kt$

Where $C_0$ is the initial concentration of Niclosamide, $C_t$ is the time dependent concentration, t is the time and $k$ is the reaction rate constant

Different variables affecting degradation rate of Niclosamide including, drug concentration, NaOH normality and temperature have been studied as following:

#### 4.2.1. Effect of Nicloamide concentration

Two different concentrations $3.06 \times 10^{-3}$ mol/L and $1.53 \times 10^{-3}$ mol/L of Niclosamide were used for the study. **Fig. 6** is a plot of logarithm of the remaining concentration of Niclosamide versus time. Results in **Table 4** shows that the slope of the obtained curves was nearly the same. The rate of the degradation ($k$) was almost the same for

ACCEPTED MANUSCRIPT

different concentrations of Niclosamide, and the half life time were calculated from the equations [20]:

$\ln C_t/C_0 = -kt$

$t_{1/2} = 0.693/k$

#### 4.2.2. Effect of NaOH concentration

To study the effect of NaOH concentration on the reaction rate, experiments were performed using 0.5 N and 1 N NaOH solutions. **Fig. 7** shows that the reaction rate was increased by increasing the NaOH concentration and the results are shown in **Table 5**.

#### 4.2.3. Effect of temperature

The kinetic study of Niclosamide was performed as a function of temperature where the previous procedure was applied using two different thermostatically controlled water baths set at 60 °C and 80 °C. The influence of temperature on the reaction rate constant was given by Arrehenius equation as follows [20]:

$\ln k = \ln A - E_a/RT$

Where $k$ is the reaction rate constant, A is the frequency factor, $E_a$ is the activation energy, R is the universal gas constant and T is the absolute temperature, **Fig. 8** shows that increasing the temperature leading to increasing of the reaction rate constant, while the results in **Table 6** shows that the activation energy of the reaction is the same at different temperature.

## Conclusion

The present work provides sensitive, accurate and selective double divisor-ratio spectra spectrophotometric method for the determination of Niclosamide in presence of its degradation products in bulk powder or in pharmaceutical formulations. The developed spectrophotometric method was utilized for determination of Niclosamide degradation kinetic and all variables affecting degradation rate ($k$) were studied. The hydrolysis of Niclosamide obeyed a pseudo-first order kinetics. It was observed that the degradation rate constants of Niclosamide were increased as a function of

ACCEPTED MANUSCRIPT

increasing the alkaline concentration or the temperature while it was almost the same at different concentrations of Niclosamide.

**References**

1- Merk Index, 13th ed., B. Susan (Ed),Merk Research Laboratories Division Of Merk and Co., Inc., Whitehouse Station, NJ,(2001), 607.

2- The British Pharmacopoeia, BP, British Pharmacopoeial Comission, London. (2009).

3- Rang and Dale. Pharmacology. 5th ed. Churchill Livingstone; p. 690.

4- Goodman and Gilman's. The pharmacological basis of therapeutics. 10th ed. McGraw-Hill; p. 1133

5- F. Onur, N. Tekin, Spectrophotometric determination of Niclosamide and Thiabendazole in tablets, Anal Lett; 27(12), (1994), 2291-30.

6- H. G. Daabees, Selective differential spectrophotometric methods for determination of Niclosamide and Drotaverine hydrochloride, Anal Lett; 33(4), (2000), 639-56.

7- S.A. Abdel fattah, Spectroscopy letters, Sectrophotometric Determination of Niclosamide using P-Benzoquinine, 30 (5), (1997), 795-804.

8- M. Algarra, B. B. Campos, J. E. Rodriguez-Borges, F. G. Sánchez, J. M. López-Romero, J. M. López-Romero, J. C. G. Esteves da Silva, Niclosamide quantification in methyl-β-cyclodextrin after derivatization to aminoniclosamide; Journal of Inclusion Phenomena and Macrocyclic Chemistry, February, 72 (1-2), , (2012), 89-94.

9- T. M. Schreier, V. K. Dawson, Y. Choi, N. J. Spanjers, M. A. Boogaard, Determination of niclosamide residues in rainbow trout (Oncorhynchus mykiss) and channel catfish (Ictalurus punctatus) fillet tissue by high-performance liquid chromatography, J Agric Food Chem, 48(6), (2000), 2212-5.

ACCEPTED MANUSCRIPT

10- Y. Wu, D. Gong, X. Peng, H. Xie, B. Han, Determination of niclosamide ethanolamine residue in rice and paddy field by high performance liquid chromatography, Se Pu. 29(11), (2011), 1098-102.

11- H. Jiang, Y. Zhang, X. Chen, J. Lvb, J. Zou, Simultaneous determination of pentachlorophenol, niclosamide and fenpropathrin in fishpond water using an LC-MS/MS method for forensic investigation, Anal. Methods, 5 (1), (2013), 111-115.

12- C. G. Derek-Muira, N. P. Grift, Determination of Niclosamide (Bayer 2353) in Water and Sediment Samples, International Journal of Environmental Analytical Chemistry, 8 (1), (1980).

13- H. Alemu, N. M. Khoabane, P. F. Tseki, Electrochemical oxidation of niclosamide at a glassy carbon electrode and its determination by voltammetry, Bulletin of the Chemical Society of Ethiopia, 17 (1), (2003), 95-106.

14- H. Alemu, P. Wagana, P. F. Tseki, Voltammetric determination of niclosamide at a glassy carbon electrode. Analyst. 127(1), (2002), 129-34.

15- "European pharmacopoia", Council of Europe European (COE)- European Directorate for the Quality of Medicines (EDQM); 2007.

16- J.J. Espinosa-Aguirre, R.E . Reyes, C Cortinas de Nava, Mutagenic activity of 2-chloro-4-nitroaniline and 5-chlorosalicylic acid in *Salmonella typhimurium*: two possible metabolites of niclosamide, Mutation Research Letters, 264 (3), (1991), 139-145.

17- www.sigmaaldrich.com

18- E.R. Garrett, R.F. Carper, Prediction of stability in pharmaceutical preparations I Color stability in a liquid multisulfa preparation, j. Am. Pharm. Assoc. 44, (1955), 515.

ACCEPTED MANUSCRIPT

19- D. A. Cairnes, W. E. Evans. Ther. Drug Monit. 5, (1983), 363-366.

20- A. Martin, J. Swarbrick, A. Cammarata, physical pharmacy, 3 rd edn., Lea and Febiger, Philadelphia, (1983), pp 359-360.

21- E. Dinc. The spectrophotometric multicomponent analysis of a ternary mixture of ascorbic acid, acetyl salicylic acid and paracetamol by the double divisor-ratio spectra derivative and ratio spectra zero crossing methods, Talanta.48,(1999), 1145-115.

22- E. M. Hassan, M. S. Mahrous, R. N. Shdeed, Spectrophotometric determination of the active components of a ternary mixture containing chlorpheniramine maleate, diclofenac sodium and paracetamol, J. Pharm. Bio. Sci,7 (06), (2011),1-9.

23- N. S. Abdelwahab, E. A. Abdelaleem, Application of fourier function to double divisor ratio spectra curves for analysis of some Amoebicide drugs in their ternary mixtures, J. Pharm.Anal.ACTA, 3 (2), (2012).

24- The United States Pharmacopoia and National Formulary. The official Compendia of Standards, Asian Edition, USP 30-NF 25 The United State

25- Y. X. Guo, Z. L. Xiu, D. J. Zahang, H. Wang, L. X. Wang, H. B. Xiao,Kinetics and mechanism of degradation of Lithospermic acid B IN aqeous solution, J. Pharm. Bio. Anal, 43, (2007),1249-1255.

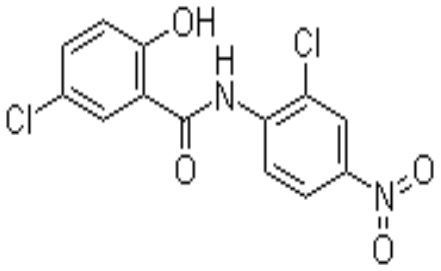
26- S. M. Sabry, M. Abdel-Hady, M. Elsayed, O. T. Fahmy, H. M. Maher, Study of stability of Methotrexate in acidic solution Spectrofluorimetric determination of Methotrexate in pharmaceutical preparations through acid-catalyzed degradation reaction, J. Pharm. Bio. Anal, 32, (2003),409-423.

ACCEPTED MANUSCRIPT

27- H. E. Zaazaa, S. S. Abbas, M. Abdelkawy, M. M. Abdelrahman, Spectrophotometric and spectrodensitometric determination of Clopidogrel Bisulfate with kinetic study of its alkaline degradation,Talanta, 78,(2009), 874-884.

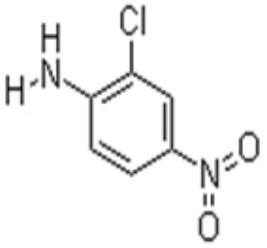
ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

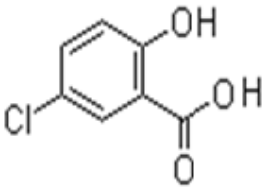


**Niclosamide**
**Mol. Formula**
**$C_{13}H_8Cl_2N_2O_4$**
**(Mol. Wt. 327)**

Reflux for 6 h using 1 N NaOH



+



**DEG I**
**2-chloro-4-nitro aniline**
**Mol. Formula $C_6H_5ClN_2O_2$**
**(Mol. Wt. 173)**

**DEG II**
**5-chloro salicylic acid**
**Mol. Formula $C_7H_5ClO_3$**
**(Mol. Wt. 173)**

**Fig. 1.** Degradation pathway of Niclosamide.

ACCEPTED MANUSCRIPT

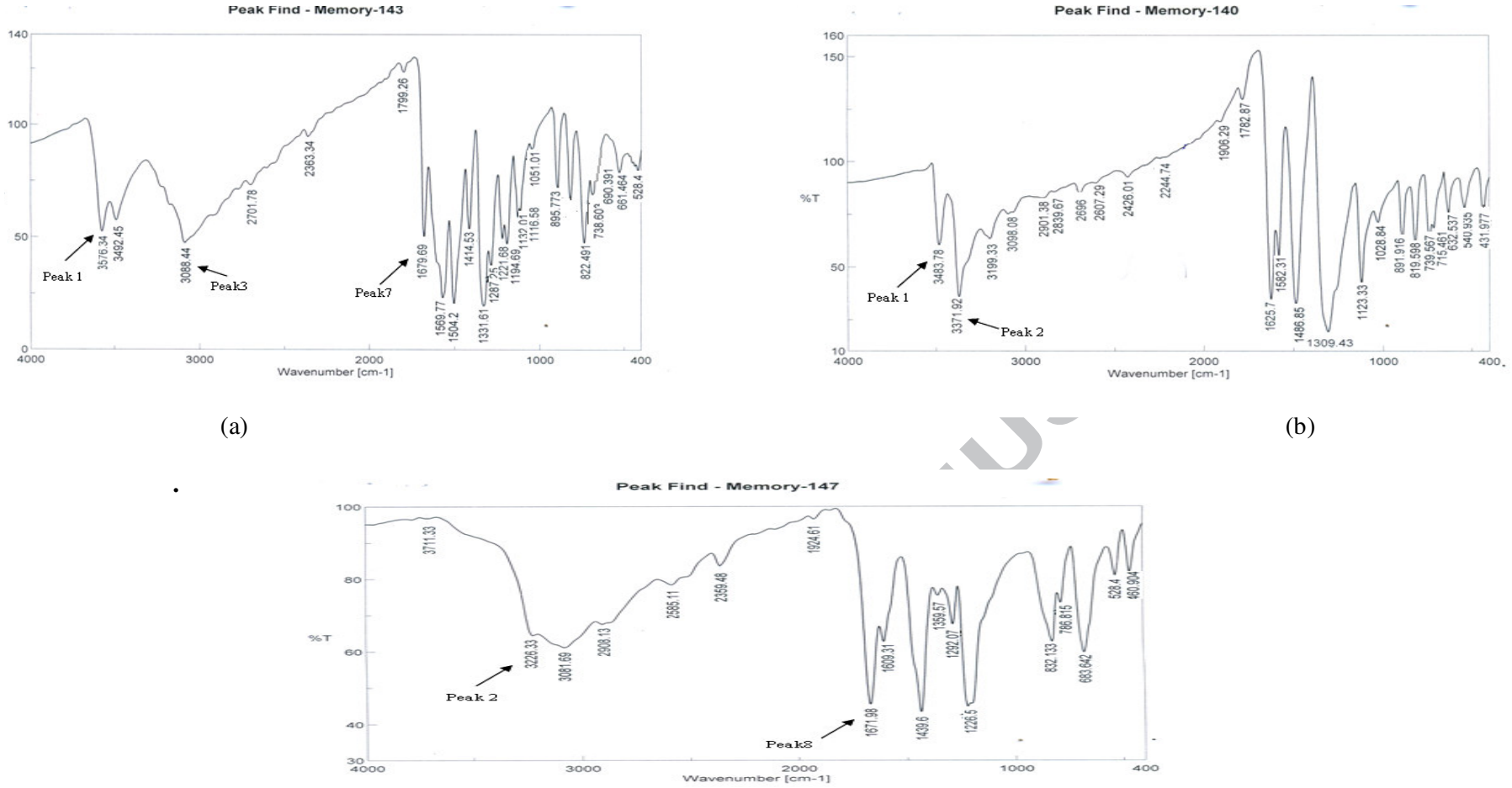
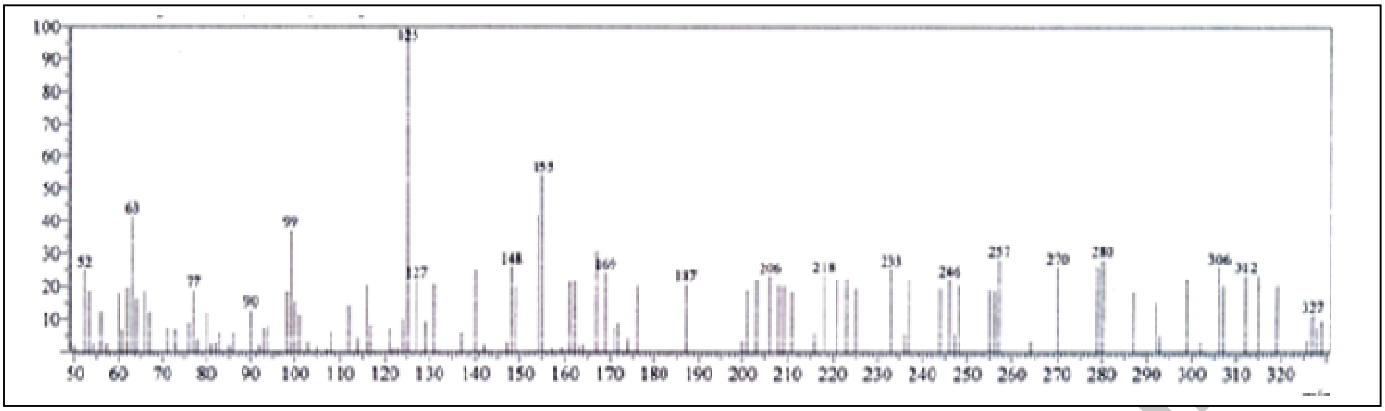
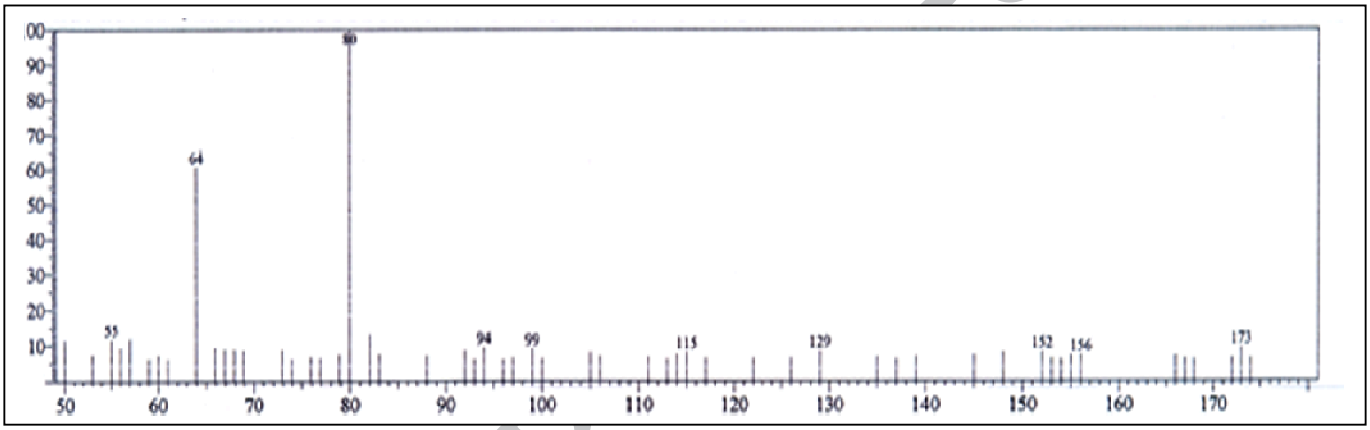



**Fig.2.** IR spectrum of (a)Niclosamide, (b) DEG I and (C) DEG

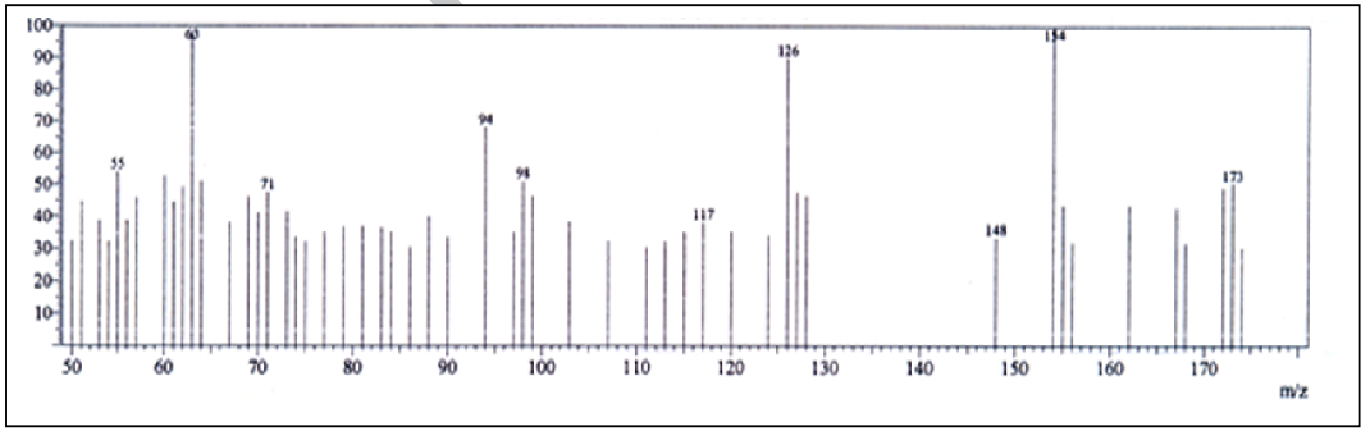
ACCEPTED MANUSCRIPT



(a)



(b)



(c)

**Fig. 3.** Mass spectrum of (a) Niclosamide, (b) DEG I and (c) DEG II

ACCEPTED MANUSCRIPT

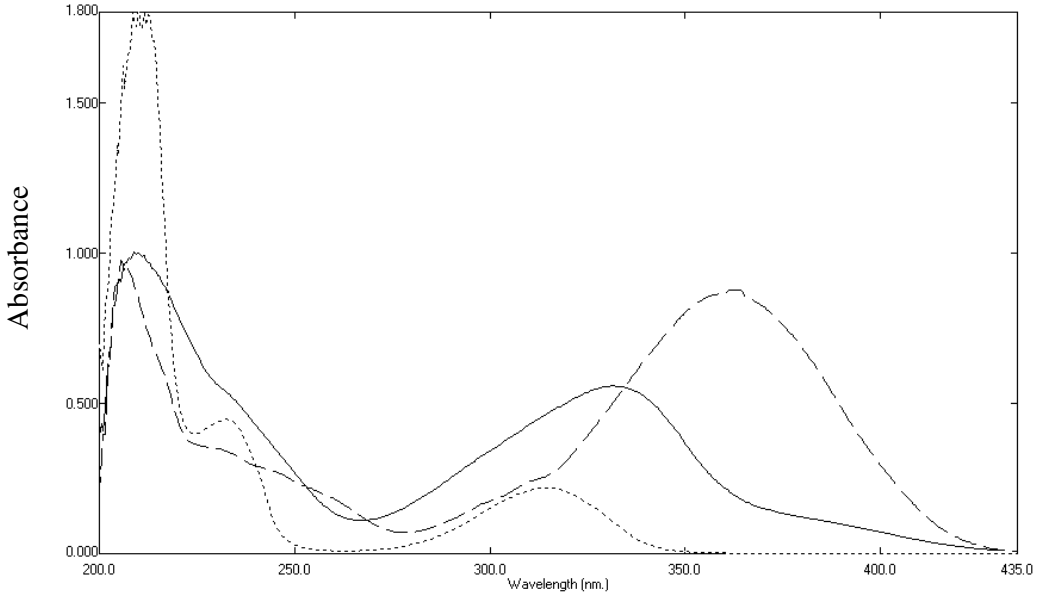



**Fig. 4**: Zero order absorption spectrua of 10 µg/mL of each of Niclosamide (_), Deg I(----) and Deg II (….) using methanol as a solvent.

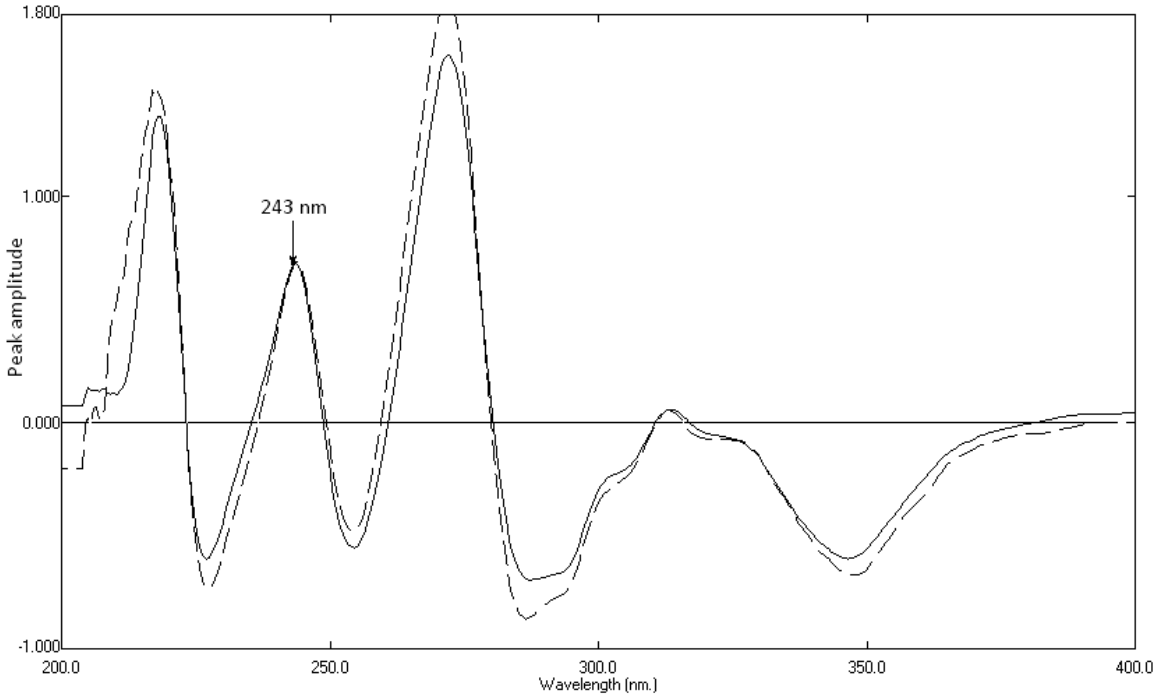



**Fig. 5:** The coincident spectra of the first derivative of the ratio spectra of 18 µg mL-1 pure Niclosamide (—) and a ternary mixture (18 µg/mL Niclosamide + 6 µg/mL Deg I + 6 µg/mL Deg II) (-----) using 10 µg/mL of each Deg I and Deg II as a double divisor.

ACCEPTED MANUSCRIPT

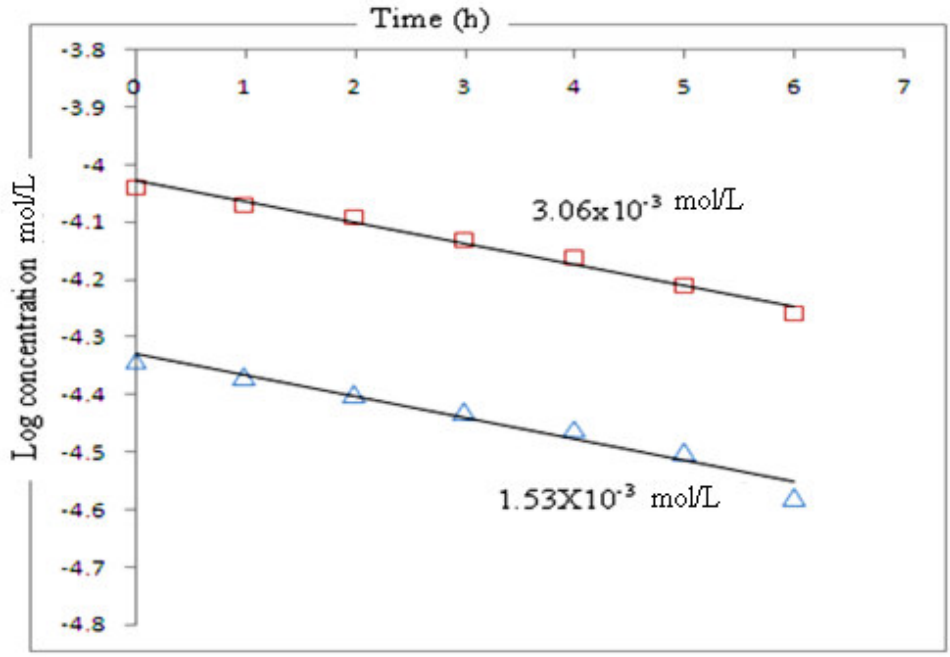



**Fig. 6:** kinetic plot of the alkaline degradation of $3.06x10^{-3}$ mol/L and $1.53x10^{-3}$ mol/L of Niclosamide using 1 N NaOH.

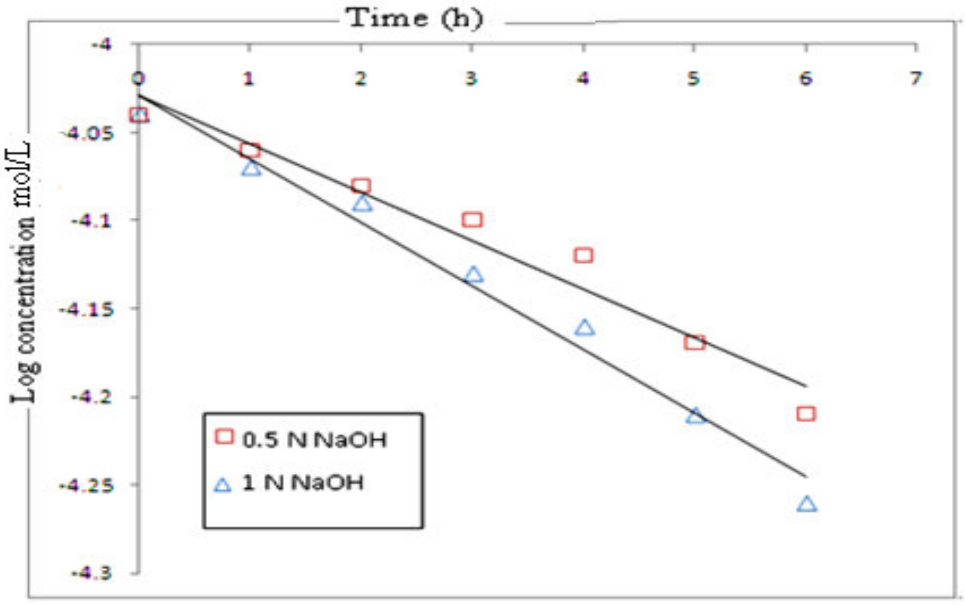



**Fig. 7:** Plot for the effect of sodium hydroxide concentration on the rate of degradation of $3.06x10^{-3}$ mol/L of Niclosamide.

ACCEPTED MANUSCRIPT

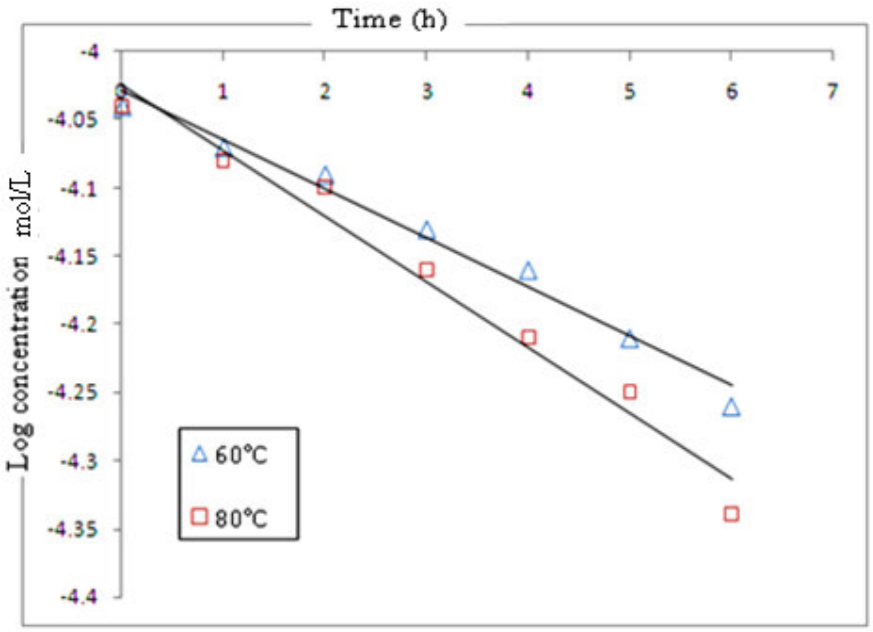



**Fig. 8:** Plot for the effect of temperature on the rate of degradation of $3.06 \times 10^{-3}$ mol/L of Niclosamide.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

**Table 1**: Determination of Niclosamide in presence of its alkaline degradation products in laboratory prepared mixtures by the proposed double divisor-ratio spectra spectrophotometric method.

| % Degradation products * | Concentration (µg/mL) | | | Double divisor-ratio spectra spectrophotometric method Recovery %** |
|---|---|---|---|---|
| | Niclosamide | DEG I | DEG II | Niclosamide at 243 nm |
| 0.99 | 30 | 0.15 | 0.15 | 102.63 |
| 4.11 | 28 | 0.6 | 0.6 | 99.61 |
| 8.45 | 26 | 1.2 | 1.2 | 100.19 |
| 15 | 25 | 2.5 | 2.5 | 101.16 |
| 30 | 21 | 4.5 | 4.5 | 101.48 |
| 40 | 18 | 6 | 6 | 99.83 |
| 50 | 15 | 7.5 | 7.5 | 98.33 |
| 65 | 10 | 10 | 10 | 102.70 |
| **Mean ± SD** | | | | 100.74±1.528 |

* The concentration of the two degradation products is according to their molecular weight.
** Average of 3 determinations.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

**Table 2**: Results of assay validation parameters of the proposed method for determination of Niclosamide.

| **Parameters** | **Double divisor-ratio spectra spectrophotometric method** |
|---|---|
| Range (µg/mL) | 4-30 |
| Slope<br>Intercept<br>Correlation coefficient | 0.0369<br>0.0217<br>0.9997 |
| Accuracy<br>(mean ± SD) | 100.14±1.283 |
| Selectivity<br>(mean ± SD) | 100.74±1.528 |
| Precision (%RSD)<br>Repeatability*<br>Intermediate precision* | <br>1.31<br>1.67 |
| LOD** (µg/mL) | 0.93 |
| LOQ** (µg/mL) | 2.78 |

* The intraday precision (n=3), average of three different concentrations (6, 8 and 10 µg/mL) repeated three times within day. The interday precision (n=3), average of three different concentrations repeated three times in three successive days.
** Limit of detection and quantitation are determined via calculations LOD = (SD of the response/slope) × 3.3; LOQ = (SD of the response/slope) × 10.

ACCEPTED MANUSCRIPT

**Table 3:** Statistical comparison of the results obtained by the double divisor-ratio spectra spectrophotometric method and the reported spectrophotometric method for the determination of Niclosamide in its pharmaceutical formulations.

| Items | Yomeasn® tablets | | Niclosan® tablets | |
|---|---|---|---|---|
| | Double divisor-ratio spectra spectrophotometric method | Reported method [5]* | Double divisor-ratio spectra spectrophotometric method | Reported method [5]* |
| **Mean** | 98.00 | 97.17 | 96.26 | 96.17 |
| **SD** | 1.252 | 1.456 | 1.523 | 1.472 |
| **Standard addition technique **(Mean± SD)** | 100.86± 1.077 | - | 99.83± 1.230 | - |
| **%RSD** | 1.277 | 1.498 | 1.582 | 1.531 |
| **N** | 6 | 6 | 6 | 6 |
| **Variance** | 1.567 | 2.119 | 2.319 | 2.167 |
| **Student's t-test** (2.228) *** | 1.067 | - | 0.109 | - |
| **F-value** (5.050) *** | 1.352 | - | 1.078 | - |

* Spectrophotometric determination of Niclosamode by measuring the ΔA values at 405.8 nm

** Average of 3 determinations .

******* Figures between parenthesis represent the corresponding tabulated values of t and F at p= 0.05.

ACCEPTED MANUSCRIPT

**Table 4**: kinetic data for the effect of Niclosamide concentration on its alkaline degradation process.

| Concentration (mol) | K (mol/h) | $t_{1/2}$ (h) | Regression equation |
|---|---|---|---|
| $3.06X10^{-3}$ | 0.0829 | 8.35 | Y=0.037C-4.0289 r=0.9798 |
| $1.53X10^{-3}$ | 0.0825 | 8.13 | Y=0.036C-4.3286 r=0.9899 |

**Table 5**: kinetic data for the effect of sodium hydroxide concentration on the alkaline degradation of Niclosamide.

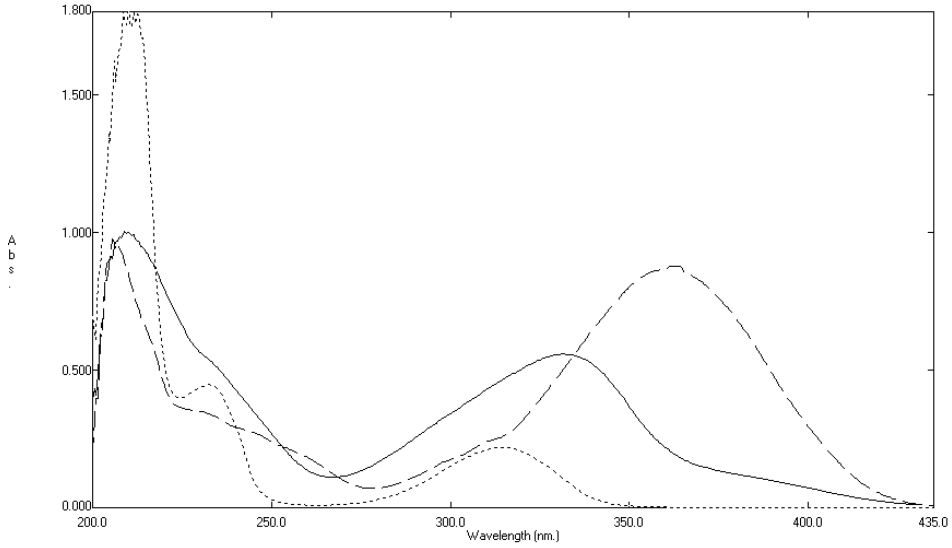
| Sodium hydroxide concentration | K (mol/h) | $t_{1/2}$ (h) | Concentration (mol) |
|---|---|---|---|
| 1N NaOH | 0.0829 | 8.35 | $3.06X10^{-3}$ |
| 0.5 N NaOH | 0.0622 | 11.14 | $3.06X10^{-3}$ |

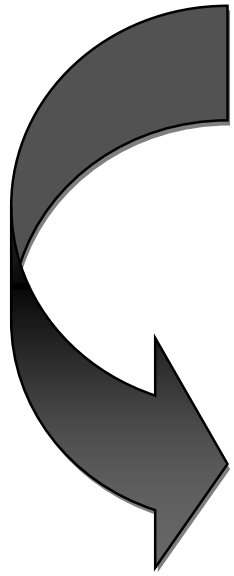
**Table 6**: Activation energy, Frequency factor and half life for Niclosamide in solution of 1N NaOH.

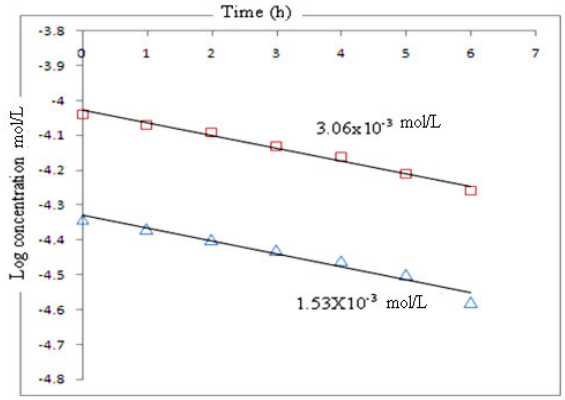
| Temperature | K (mol/h) | $t_{1/2}$ (h) | $E_a$ kcal/mol | A |
|---|---|---|---|---|
| 60°C | 0.0829 | 8.35 | 3.41 | 0.084 |
| 80°C | 0.111 | 6.24 | 3.41 | 0.113 |

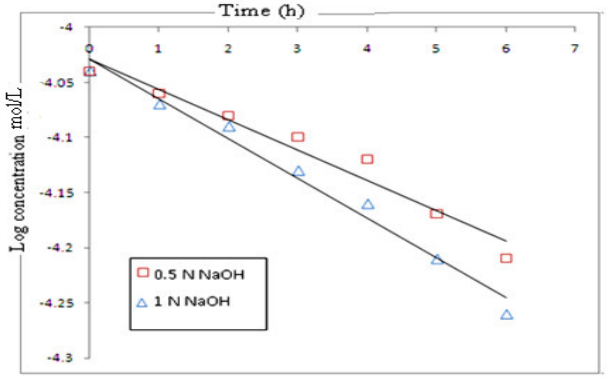
ACCEPTED MANUSCRIPT

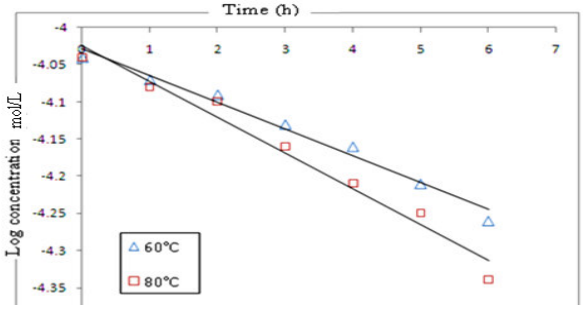
ACCEPTED MANUSCRIPT





ACCEPTED MANUSCRIPT

1. No kinetic spectrophotometric study has been reported for the assay of Niclosamide
2. spectrophotometric kinetic study of Niclosamide alkaline degradation as a function of drug concentration, alkaline concentration and temperature has been established utilizing double divisor-ratio spectra spectrophotometric method.
3. Niclosamide followed pseudo-first order under with a degradation rate constant ($k$) of 0.0829 mol/h and half life ($t_{1/2}$) of 8.35 h
4. The overall degradation rate constant as a function of the temperature under the given conditions obeyed Arrehenius equation where the activation energy was calculated to be 3.41 kcal/mol.

ACCEPTED MANUSCRIPT