MERIT RESEARCH JOURNALS
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Merit Research Journal of Medicine and Medical Sciences (ISSN: 2354-3238) Vol. 3(2) pp. xxx-xxx, February, 2015
Available online http://www.meritresearchjournals.org/mms/index.htm

Original Research Article

Stability-indicating Colorimetric method for the analysis of Niclosamide

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Abstract

Simple, accurate and stability-indicating colorimetric method was developed for the determination of niclosamide in bulk and dosage forms. The method was based on the alkaline hydrolysis of niclosamide with 0.1M sodium hydroxide under the specified reaction conditions. The zero-order and first derivative spectra of the degradation product (yellow colored) were recorded at 380nm and 409nm respectively. Linearity was exhibited in the concentration range 4-20µg/ml with good correlation coefficient (not less than 0.999). The recovery percentage results (99.47 ± 1.30%, n=3) indicated the absence of interference by the tablets recipients. The results obtained by the developed method were statistically compared by the results of a reported method for determination of niclosamide, and evaluated at 95% confidence limits.

Keyword: Colorimetry, Degradation product, Derivative spectrophotometry, Niclosamide, Zero-order

INTRODUCTION

Niclosamide (Figure 1) is orally administered anthelmintics drug. It is highly effective against cestodes infesting man-Taenia saginata, T.solium, Diphyllobothrium latum and Hymenolepis nana, as well as 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 partly digested in the intestine (Tripathi, 2003; Rang and Dale, 2005; Goodman and Gillman's, 2001).

Recent reports claimed niclosamide as anticancer agent. Niclosamide reported to inhibits the Wnt/βcatenin, mTORC1, STAT3, NF-κB and Notch signaling pathways, and induce cell cycle arrest, growth inhibition and apoptosis (Li Y et al, 2014).

Various methods had been reported for the analysis of niclosamide in bulk and dosage forms. These methods include stability-indicating methods (Shaza et al., 2015), chromatographic and spectrophotometric methods (Daabees, 2000; Cholifah et al., 2008; Feyyaz and Nigar, 1994)

In the present work, a simple stability-indicating colorimetric method was developed for the determination of niclosamide in bulk and dosage form.

Experimental/Instrumentation

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

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. Sodium hydroxide (BDH), Pool, England; Methanol Scharlau Chemie S.A., Spain; Acetone Scharlau Chemie S.A., Spain.

Standard stock solution

NA standard solution (2.5 mg/ml) was freshly prepared i n acetone. 1ml was further diluted with methanol to obtain

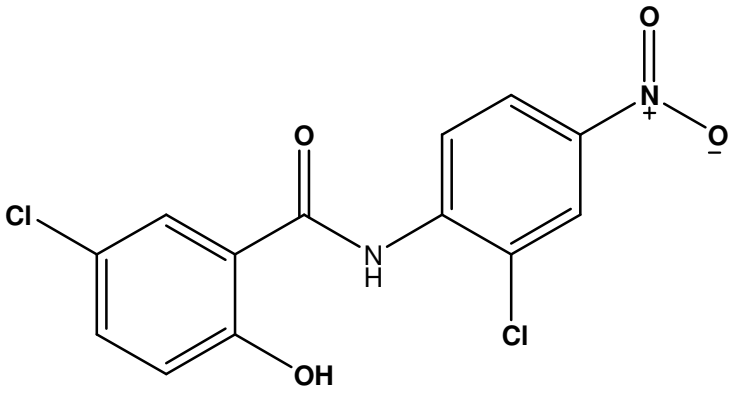

Figure 1. Chemical structure of Niclosamide

200µg/ml solution (solution A).

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 200µg/ml solution (solution B).

Procedures

Calibration Curve

Different volumes from solution A (200µg/ml) containing 40-200µg/ml were transferred to a set of round bottom flasks. 1ml of 0.1M NaOH was added to each flask. The volumes were then completed to 10ml with methanol. The flasks were connected to condensers and heated in a boiling water bath for 30 minutes. The reaction was quenched by cooling; the reaction mixture was then transferred into 10ml volumetric flask and volume adjusted to 10ml if needed. Zero and first order derivative spectra were then recorded over the range 310-450nm for each solution. The obtained absorbance values were then plotted against concentration and the regression analysis data was obtained from the plot.

For determination of tablet content, 3ml of solution B was treated as under calibration curve. The sample content was then calculated from the plot or by direct sample/ standard comparison.

Precision and Recovery studies

Within-day and between-day data were determined for four concentrations within the linearity range. The relative standard deviation (RSD) values were then calculated as an evaluation for the precision of the method.

The percent recovery of the method was tested through conducting the following experiment: 0.4ml of solution A was transferred to round bottom flask. 0.4ml of solution B was then added to the flask. 0.4ml of either the sample or the standard stock solutions was transferred to separate round bottom flasks. The three solutions were treated as under the calibration graph. The absorbance of each solution was measured over the range 310-450nm.The percentage recovery was calculated using the following formula (Shantier et al., 2011):

$$\frac{A_T - A_{Sm}}{A_{Std}} \times 100$$

Where A_T = total absorbance of mixture (tablet solution + authentic solution), A_{Sm} = absorbance of sample solution A_{Std} = absorbance of authentic solution.

RESULTS AND DISCUSSION

For any drug stability studies, a validated stability-indicating method that can distinguish the active ingredient from its degradation products is the first and major requirement by the International Conference on Harmonization (ICH) (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use, 2013).

Direct spectrophotometric methods (zero-order) are widely used in pharmaceutical analysis although they lack selectivity. Prior to the development of derivative spectroscopy, spectrophotometry did not prove in most cases to be a useful tool in stability-indicating procedure. Colorimetric methods are sometimes utilized as stability-indicating methods by selectively transforming a drug, its degradation product or its impurity into a derivative so that the spectrum of the derivative is shifted to the visible region (Mehta, 1995).

The zero-order and first derivative spectra of NA showed absorption maxima at 332nm and 351nm

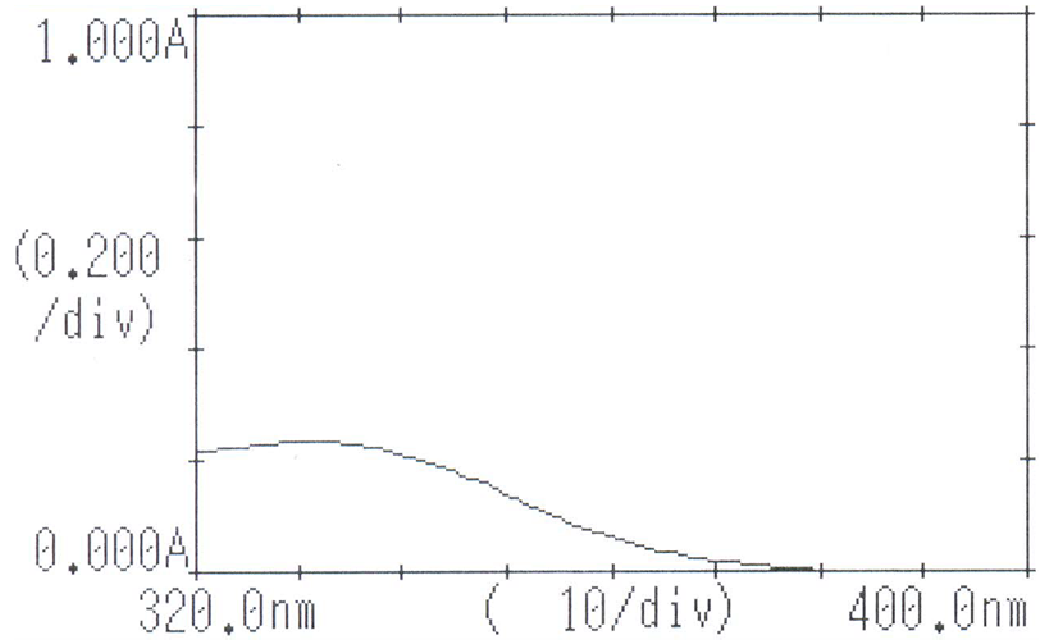

Figure 2. Zero order spectrum of niclosamide (6µg/ml, 332nm)

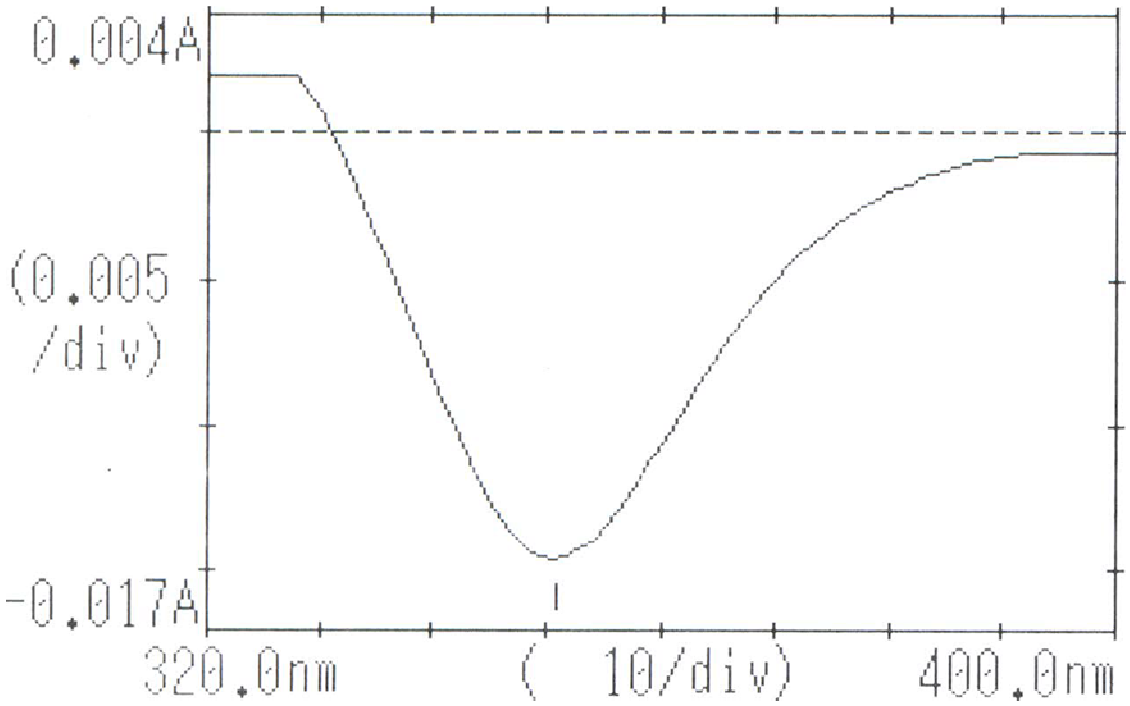

Figure 3. First derivative spectrum of niclosamide (6µg/ml, 351nm)

Respectively (Figures 2, 3).

During the stability studies on niclosamide, the formation of a faint color was noticed when 0.1N NaOH was added to the solution of niclosamide. The intensity of the color was observed to increase with heating. This observation tempted the authors to study the direct reaction of niclosamide with 0.1M NaOH and to investigate the possible use of the reaction as a direct quantitative and stability-indicating method.

Upon the addition of 0.1M sodium hydroxide, the zero-order and first derivative spectra of niclosamide solution reflected a decrease in niclosamide peak at its maximum

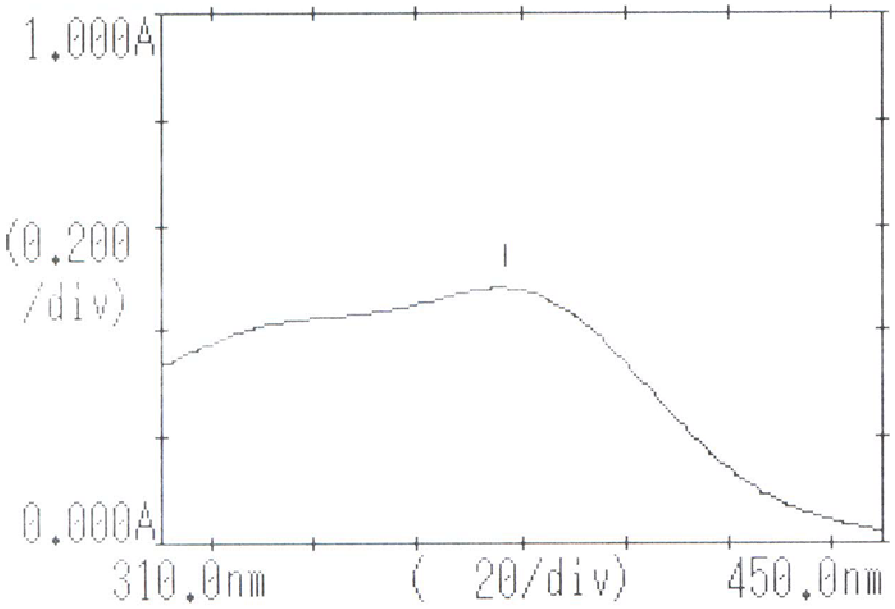

Figure 4. UV/VIS spectrum of the degradation product (6µg/ml, 380nm)

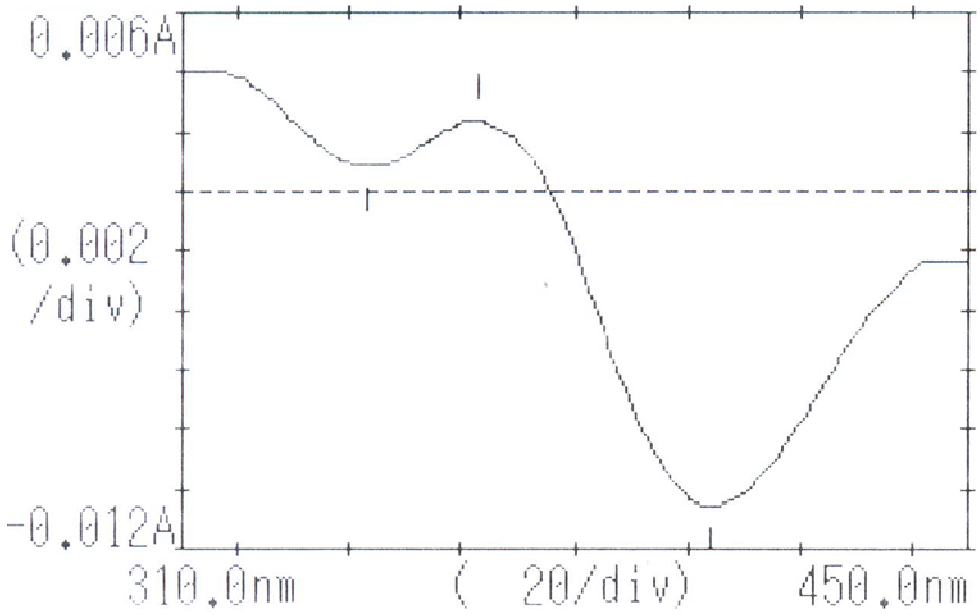

Figure 5. First derivative spectrum of degradation product (6µg/ml, 409nm)

wavelengths and the formation of yellow colored degradation product with λ_{max} at 380 nm and 409 nm respectively.

Figures 4 and 5 show the corresponding zero-order and first derivative spectra for niclosamide after heating with sodium hydroxide for 30 minutes at a boiling water bath. The peak at 332nm disappeared as a result of the C-N bond cleavage (figure 1); with subsequent formation of niclosamide degradation product absorbing at 380nm (zero-order); 409nm (first derivative)

Analytical curve

Factors like sodium hydroxide normality and volume coupled with the reaction time and temperature that can quantitatively hydrolyse the drug were studied and optimized.

The formed product was found to remain stable for at least 24 hr. The optimized conditions were utilized to construct the calibration curve relating the niclosamide concentration in a range 4-20µg/ml to the absorbance

Table 1. Linearity data of the proposed method

Parameter	Developed method	
	Zero-order	First derivative
Slope	0.034 ± 0.0016	$0.00078 \pm 9.8 \times 10^{-5}$
Intercept	0.0025 ± 0.021	$0.00019 \pm 1.3 \times 10^{-3}$
Correlation coefficient	0.9998	0.9987
Range	4-20µg/ml	4-20µg/ml
LOD	0.56µg/ml	1.5µg/ml
LOQ	1.88µg/ml	5.0µg/ml

Table 2. Validation results of Developed Method compared to the Reported Differential method

	%± SD	*t cal, t(tab)	*F cal, F(tab)
Colorimetric method	100.30 ± 0.83	0.77 (2.78)	1.19 (19)
Reported method	99.80 ± 0.76	-	-

Table 3. Reproducibility and precision data as evaluated by RSD%

Concentration µg/ml	Within-day (n=3) RSD%	Between days (n=3) RSD%
8	1.5	1.5
12	0.69	1.4
16	1.8	1.5
20	1.3	0.4

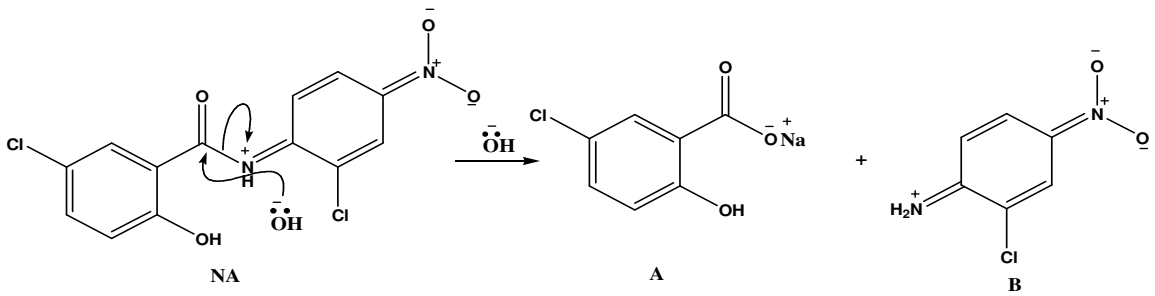

Figure 6. Proposed pathway for niclosamide degradation

values at 409nm. The regression analysis data was calculated at 95% confidence level for the developed methods using the following formula (Miller and Miller, 2005):

$y = (b \pm ts_b)\, x + (a \pm ts_a)$

Where b is the slope, a the intercept, s_b standard deviation of slope, s_a standard deviation of intercept, the t-value at 95% confidence level for (n – 2).

The results obtained for linearity data of the proposed method reflected the accuracy and consistency of the curve Table 1.

Assay and Validation

The %± SD data for niclosamide assay using the proposed method was 100.30 ± 0.83%; n=3, which reflected the accuracy of the method.

It was not possible to validate the developed method using the official method due to some technical difficulties. Instead, the validity was assessed by comparing the statistical results obtained with those of a reported method (Daabees, 2000).

Data of Table 2 show the obtained assay results and the calculated *t*- and *F*-values as compared to the corresponding tabulated values at 95% confidence level. As the calculated *t*- and *F*-values were less than tabulated ones, the result of this method can be considered as accurate and precise as the reported method. The developed method can be recommended for drug determination in quality control and routine analysis of the drug.

Recovery and Precision

The freedom of interference by the tablet excipients was confirmed by the good results of the added recovery using the proposed method (99.47 ± 1.30%, n=3).

The obtained results for reproducibility and repeatability of the proposed method were summarized in table 3. The results obtained show good reproducibility and precision which are reflected by the low RSD values.

Proposed Reaction Pathway

The alkaline hydrolysis of niclosamide was found to resemble the metabolic hydrolytic cleavage of niclosamide to chlorosalicylic acid (Compound A) and 2-chloro-4-nitroaniline (Compound B). It had been suggested that 2-chloro-4-nitroaniline is the yellow colored product because of the extended conjugation (Shaza et al., 2015). (Figure 6)

CONCLUSION

Simple, sensitive and accurate colorimetric method was developed for the determination of niclosamide in bulk and dosage forms. The developed method can be adopted as stability-indicating method for the analysis of niclosamide.

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