Journal Of Harmonized Research (JOHR)

Journal Of Harmonized Research in Pharmacy
4(1), 2015, 87-92

ISSN 2321 – 0958

Original Research Article

DEVELOPMENT OF DERIVATIVE SPECTROPHOTOMETRIC AND HPLC METHODS FOR DETERMINATION OF NICLOSAMIDE

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Abstract: Simple, sensitive and accurate derivative spectrophotometric and HPLC methods were developed for the determination of niclosamide in bulk and dosage forms. The spectrophotometric method was based on the measurement of the first derivative spectrum for the methanolic solution of niclosamide at λ_{max} 351nm. The HPLC-separation was conducted on Shimpac C-18 (25x4.6mm) column using a mobile phase consisted of 70: 30 v/v methanols: water. System suitability was assessed by measurement of factors affecting column efficiency. Beer's law was obeyed over the concentration range 2-12µg/ml with a correlation coefficient not less than 0.999. The added recovery results were 100.80 ± 0.59 (n=3), which indicates the absence of interference by the tablets excipients. The results obtained by the developed methods for the tablet dosage form were statistically compared with those of a reported method and evaluated at 95% confidence limits.

Keywords: Derivative spectrophotometry, HPLC, Niclosamide tablets.

Introduction:

Niclosamide (NA) is an orally administered antihelmintic drug. It is 5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide (Fig. 1). It is a yellowish grey powder, soluble in acetone and sparingly soluble in methanol [1].

Literature survey reveals various spectrophotometric and chromatographic methods for the determination of niclosamide in bulk and dosage form [2-5].

For Correspondence:
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Received on: February 2015
Accepted after revision: March 2015

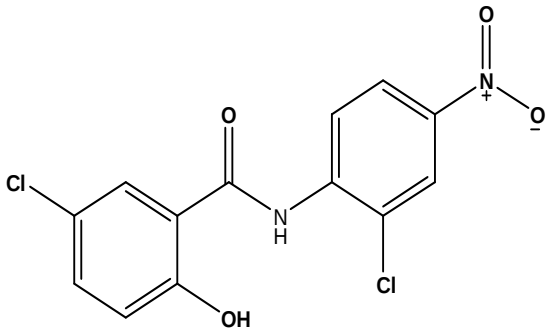

Figure 1: Chemical structure of niclosamide

The present work describes new spectrophotometric and HPLC methods developed for the determination of NA in bulk and dosage forms.

Materials and Methods

Apparatus

UV spectrophotometric studies were carried out on Shimadzu UV-1800ENG240V, (Koyoto, Japan). HPLC analysis was carried in Shimadzu liquid chromatograph, wavelength 254nm.

Chromatographic conditions

Shimadzu liquid chromatograph was used. The column used was Shimpack VP-ODS (250 x 4.6mm). The detector was SPD- 20A prominence UV/VIS. The mobile phase used is methanol: water (70:30 v/v) at a flow rate 1ml min^{-1}. Ultraviolet setting was at 254nm and 20µl volumes were injected onto the column at room temperature.

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. Methanol Scharlau Chemie S.A., Spain.

Preparation of solutions

Standard stock solution

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

Sample preparation

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

Procedures

Calibration curve

Different accurately measured volumes (0.2-1.0 ml) of solution A were transferred to five volumetric flasks (10mL). Volumes were then completed to mark with methanol. First derivative spectrum was recorded over the range 320-400nm. Regression analysis data was obtained from the absorbance-concentration graph.

HPLC chromatogram was also recorded by injecting 20µl volumes of the five solutions above.

For determination of NA tablet content, 0.4mL from each solution A and B were diluted to 10 ml as per the treatment under calibration graph experiment for both developed methods. A graph was also constructed for both solution A and B using the developed methods to compare the slopes of these graphs as another means for tablet content determination.

Mobile phase polarity index calculation

Polarity indices (P'ab) for the systems used were calculated using the following formula[6]:

$P'ab = \theta a + \theta b$

Where θ is the polarity index of the solvent, a is the fraction of aqueous phase, b is the fraction of methanol.

Recovery and Precision

The calculation of added recovery was done using an adopted formula:

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

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

Repeatability and reproducibility were determined for four different concentrations within the linearity range. The relative standard deviation values (RSD) were then calculated.

Limit of detection and quantification (LOD; LOQ)

LOD and LOQ were determined from calibration curves of the proposed methods using the adopted formulae respectively in reference [7]:

$$3SB/b \quad ; \quad 10SB/b$$

Where SB= Sy/x (calculated from the regression analysis data), b is slope.

Results and Discussion

Derivative spectroscopy is a simple powerful technique. It is suitable for analysis of turbid solutions [8], and can be used successfully for the assay of pharmaceutical formulations.

The original UV spectrum (zero-order) of NA showed a broad peak at 332nm (Fig.2). The first derivative of the zero order spectrum showed better resolved peak at 351nm (Fig.3). Therefore the first derivative spectrophotometric method was preferred for the assay of the drug in bulk and dosage form.

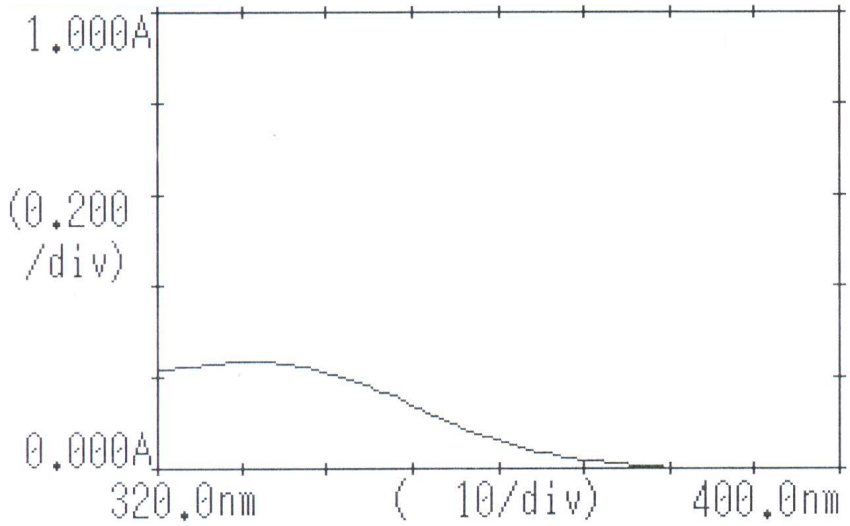

Figure 2: Zero order spectrum of niclosamide (6µg/ml, 332nm)

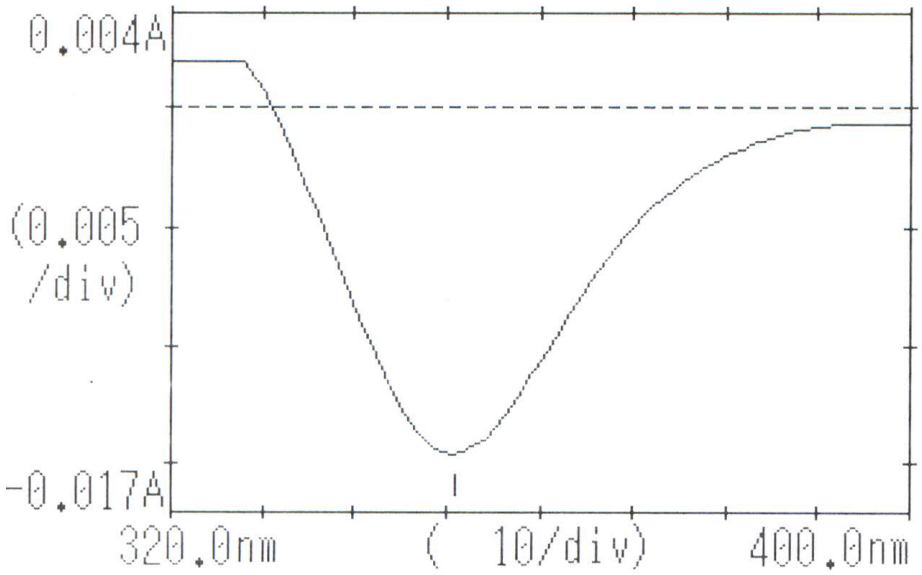

Figure 3: First derivative spectrum of niclosamide (6µg/ml, 351nm)

Selection of the mobile phase

In order to obtain a column/mobile phase system that would be suitable for the quantification of NA, different mobile phases were investigated. The column used was Shimpack C_{18} (25 x 4.6mm). The mobile phases used were composed of various percentages of organic modifiers, different pHs and different flow rates. A 50% v/v mixture of methanol and orthophosphoric acid was first investigated at flow rate 1 ml min^{-1}. This system didn't elute NA even upon using different percentages of solvent content. Another mobile phase consisting of acetonitrile and a mixture of KH_2PO_4 and Na_2HPO_4 with different percentages and flow rates was also investigated. This system eluted the drug at 2.5min but with remarkable tailing. A mobile phase consisting of 60:40 v/v methanol: acetonitrile at flow rate 0.5ml min^{-1} was also tried. NA was eluted at 6.5 min. but with a better tailing factor (1.33). The polarity index of this system was 5.38. Another system consisting of 70:30v/v methanol: H_2O resulted in a reasonable retention time (3.5min.) for the elution of NA with good peak symmetry (1.0) (Fig.4).

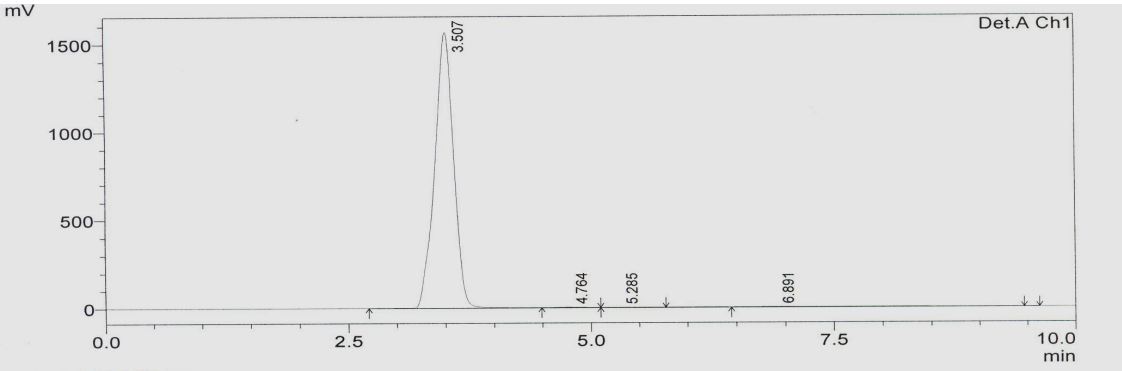

Figure 4: Typical chromatogram for standard niclosamide

The polarity index of this system was 6.63. It was found that the polarity index of the mobile phase is a sufficient criteria for good resolution and peak symmetry. In addition, the ratio of the solvent content in the mixture of the mobile phase is also important. This is confirmed by comparing results obtained for solvent mixtures of polarity 5.38 and the other solvent mixture with polarity index 6.63. The results obtained for the best system suitability parameters were summarized in Table 1.

Table 1. Results for System Suitability Criteria

Mobile phase	Flow rate ml min^{-1}	Tr	K	Peak sym.	Number of theoratical plates (N, cm-1)	HETP (mm)
60% MeOH: Acetonitrile	0.5	6.5	4.4	1.33	1056	0.024
70% MeOH:H_2O	1.0	3.5	1.5	1.00	1226	0.020

Linearity

A calibration curve was constructed using the developed methods at a concentration range 2-12µg/mL. The obtained correlation coefficient values (r) for the derivative spectroscopic and HPLC methods were 0.9996 and 0.999 respectively. The regression analysis data was calculated at 95% confidence level for the developed methods using the following formula [7]:

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

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 reflected the consistency of the prepared calibration graphs (Table 2).

Table 2. Linearity Data of the Proposed Methods

Method	λ_{max}	Slope ± ts_b	Intercept ± ts_a
Zero order	332nm	0.083 ± 0.0033	-0.004 ± 0.026
1st derivative	351nm	0.0035 ± 0.00011	0.00018 ± 0.009
HPLCmethod	254nm	53476 ± 6371	-105.7 ± 48594

Assay and Validation

The derivative spectrophotometric and HPLC methods were applied for the drug uniformity testing in Niclosamide tablets.

The reported ΔD_2 method by Daabees HG [2] was applied for the quantitative analysis of NA in bulk and dosage forms. The % ± SD (n=3) data for NA assay by the reported method was found to be 99.80 ± 0.76% (n=3).

The validity of the developed methods for the determination of NA in bulk and dosage form was assessed by comparison of the statistical

results obtained with those of the reported method.

Data of Table 3 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-value and F-value at 95% confidence limit were less than the tabulated ones, the results of the developed methods can be considered as accurate as the reported method.

Table 3. Validation of the Developed Methods Compared to the Reported Method

Method	Mean ± SD % (6µg/ml; n=3)	*t cal, t tab.	*F cal, F tab.
Derivative spectrophotometric method	101.00 ± 0.65	2.08 (2.78)	1.37 (19)
HPLC method	101.30 ± 0.95	2.14 (2.78)	1.56 (19)
Reported method	99.80 ± 0.76	-	

Recovery and Precision

The accuracy of the procedure and the absence of the interference by the tablets excipients were confirmed by the results obtained for recovery testing of added amount of standard NA to sample solution in the ratio of 1:1. The results showed good recovery (100.80 ± 0.59, n=3).

The reproducibility and repeatability of the developed spectrophotmetric method were obtained by the follow-up of within-day and between-day data for four concentrations within the linearity range. The results obtained are shown in Table 4. Good reproducibility and precision was reflected by the low RSD% values (less than 2%).

Table 4. Reproducibility and Precision Data as Evaluated by RSD%

Concentration µg/ml	Within-day (n=3), RSD%	Between days (n=3), RSD%
4	0.9	0.7
6	1.4	1.4
8	0.2	0.4
10	0.5	0.28

Limit of detection and quantification

The obtained results for LOD and LOQ were summarized in Table 5. The low values of LOD and LOQ reflected the sensitivity and suitability of the developed methods for determination of NA.

Table 5. Obtained Results for LOD and LOQ

Developed method	LOD (µg/ml)	LOQ (µg/ml)
Zero order	0.38	1.25
1st derivative spectrophotometry	0.30	1.00
HPLC	0.35	1.20

Conclusion

The developed spectrophotometric and HPLC methods were proved to be simple, sensitive, accurate and precise for the determination of NA in bulk and tablets forms. The simplicity and cost-effectiveness of the developed methods make them suitable for routine quality control analysis of Niclosamide. In addition, the major advantage of the developed methods is that the procedure does not require extraction step or great number of chemicals.

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