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Indirect Spectrophotometric Method for Determination of Niclosamide via Oxidation Reaction and Bleaching colour of Methylene Blue Dye

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Abstract:

Simple, delicate and sensitive method for estimation niclosamide (NICA) as pure, pharmaceutical and veterinary formulations. The method is based on the oxidation of niclosamide (NICA) by using an excess of the oxidizing agent N-bromosuccinimide (NBS) in an acidic medium. Then the unreacted N-bromosuccinimide is reacted with methylene blue (MB) dye where the color of the dye is bleached and measured at the maximum wavelength of the dye 664 nm. The linear graph of absorbance versus concentration indicates that Beer's law applies within the concentration range 2-6 µg.mL^{-1} of niclosamide. The determination coefficient (R^2) was found to be 0.9955 and molar absorptivity value was 4.6546x10^4 L.mol^{-1} .cm^{-1}and the Sandell's sensitivity value was *0.0*0702 µg .cm^{-2} and the quantitative limit attained (LOQ) was 0.0148 µg.mL^{-1}. The limit of detection (LOD) was 0.0044 µg.mL^{-1}. with -0.15% to -0.90% relative error and relative standard deviation was 0.65 to 1.93.

Keywords: Niclosamide, Indirect spectrophotometer, bleaching, methylene blue dye

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Introduction

Niclosamide was first synthesized in 1958[1]. It is an anthelmintic which is active against most tapeworms, including the beef tapeworm, the pork tapeworm, the fish tapeworm, the dwarf tapeworm and the dog tapeworm [2] niclosamide inhibits proliferation of colorectal cancer cells and has little to no toxicity toward nonmalignant tissues. It has the ability to induce apoptosis of cancer cells in both prostate and breast cancer cell lines [3]. Anhydrous niclosamide yellowish-white or yellowish, fine crystals, practically insoluble in water, sparingly soluble in acetone, slightly soluble in ethanol, and must be protected from light [4]. Chemical name: 2´,5-Dichloro-4´-nitrosalicylanilide; 5-Chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide molecular formula: $C_{13}H_8Cl_2N_2O_4$ =327.1 g /mol [2].

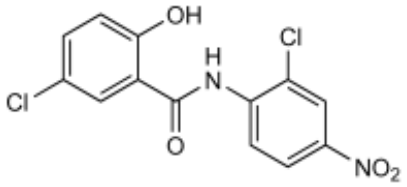

Figure 1: Chemical structure of niclosamide.

Through follow-up to scientific references, a specific number of methods for the determination of niclosamide in its pure form and in pharmaceutical preparations have been noted, below are some of these methods. Spectroscopic methods for determination of niclosamide, this method depends on the reduction of the nitro group in niclosamide by adding zinc powder in acid medium and then the reduced niclosamide interacts with other reagents such as N, N-dimethylaminobenzaldehyde in non-aqueous medium to form a colored product measured at 454 nm. [5]. Method is proposed for the determination of niclosamide include the diazotization of reduced niclosamide, then coupling with 2,6- dihydroxybenzoic acid to give a yellow-colored product which is water – soluble and has a maximum absorption at 456 nm [6]. indirect spectrophotometric method for determination of niclosamide in pharmaceutical preparations, the method is based on the oxidation of reduced form of niclosamide with iron (III) in acidic medium, then the subsequent reaction of iron (II) with 1,10- phenanthroline to produce a ferroin complex which is water – soluble, measured at 510nm[7]. Another spectral method of estimation via measuring the dA/dΔ values at 405.8 nm in the first derivative spectra of the solution for niclosamide in presence thiabendazole [8]. Colormetry method was based on the alkaline hydrolysis of niclosamide with 0.1M sodium hydroxide under the specified reaction conditions, then the zero-order and first derivative spectra of the degradation product (yellow colored) were recorded at 380 nm and 409 nm respectivel [9]. Method is based on the reaction of reduced NICA with fast red B salt in presence of ammonium chloride and sodium hydroxide [10]. Asensitive spectrophotometric method is reported for the determination of reduced (NICA) either in pure form or in formulations by reaction with metol and potassium dichromate at pH 3.0 ± 0.2 to give a coloured product having maximum absorbance at 530 nm [11]. An electrochemical sensor was fabricated with the multiplewalled carbon nanotubes/cyclodextrins composite modified glassy carbon electrode (MWCNT/CD/GCE), which was applied to analyze the niclosamide residual [12]. Cyclic voltammetry, square-wave voltammetry and controlled potential electrolysis have been used to study the electrochemical oxidation behaviour of niclosamide at a glassy carbon electrode [13]. HPLC method has been developed for simultaneous determination of anhydrous niclosamide in presece albendazole [14]. Reversed phase high performance liquid chromatographic method for the simultaneous determination of niclosamide in bulk and oral suspension for veterinary in presence albendazole [15].

The present method includes the oxidation of niclosamide NICA using an excess of the oxidizing agent N-bromosuccinimide in an acidic medium, then the unreacted N-bromosuccinamide is reacted with (MB) dye the remaining dye color is measured at the optimal wavelength of the dye 664 nm.

Experimental

Apparatus

The UV-1900i UV-Visible spectrophotometer Shimadzu (Japan) was used in spectral measurements, electronic balance KERN&Sohn GmbH (Germany) and professional Bench top pH meter BP3001(Singapore).

Chemical and solutions

All chemicals used were of analytical grade.
The niclosamide was equipped by the Italian company Sigma.
Niclosamide solution (100 µg .mL^{-1}) was prepared by dissolving (0.0100) g of NICA in absolute ethanol in a 100 mL volumetric flask and keep in a sealed dark bottle.
N-bromosuccinimide solution (1 x 10^{-3} mol/L): This solution was prepared by dissolving 0.0178 g of N-bromosuccinimide in distilled water in a 100 mL volumetric flask.
Methylene blue (MB) dye (0.01%, 3.126 x 10^{-4} mol/L): prepare this solution by dissolving 0.0100 g of dye in distilled water in a 100 mL volumetric flask.

Pharmaceutical preparation

Pharmaceutical preparation solution (100 µg. mL^{-1}): 5 tablets (each tablet contained 500 mg of niclosamide) of the medicinal product Yomesan produced by the German company Bayar Vital, were weighed, crushed and mixed

well, weighing 0.0130 g of the powder and dissolved in absolute ethanol in a volumetric flask of 100 mL after completion volume with absolute ethanol, the solution was filtered through filter paper.

Veterinary preparation solution (100 µg .mL^{-1}): 5 tablets of the veterinary preparation niclosam, produced by the Iranian Zagros Pharmed Company, were crushed and mixed well, weighing 0.0142 g (each tablet contains 1250 mg of niclosamide) and dissolved in absolute ethanol in a volumetric flask of 100 mL after completing the volume with absolute ethanol to the full extent, the solution was filtered through filter paper.

Recommended procedure and calibration curve

Prepare the calibration curve for the determination of niclosamide by adding increasing amounts of (100 µg .mL^{-1}) niclosamide solution to 10 mL volumetric flasks, 2 to 6 µg .mL^{-1} of niclosamide was added, 1 mL of N-bromosuccinimide solution (1x10^{-3} mol/L) and 1 mL of hydrochloric acid (1 M) were added to each flask, then the solutions are left for 10 minutes at room temperature, then 0.6 mL (0.01%) of MB dye was added, then waited for 15 minutes before dilution to the mark with distilled water then the absorbance is measured against the blank solution at a wavelength of 664 nm. Figure (2) represents the calibration curve for the determination of niclosamide, which follows Beer's law from 2 to 6 µg .mL^{-1}, the value of the molar absorptivity was 0.4654 x 10^{5} L.mol^{-1}.cm^{-1}, and the Sandell's significance is equal to 0.00702 µg . cm$^{-2.}$

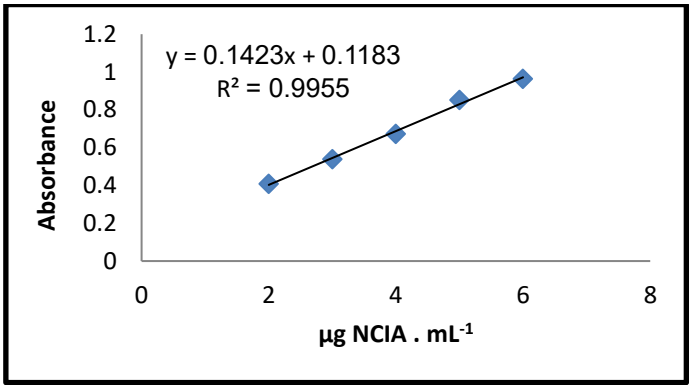

Figure 2: Calibration curve for the determination of NICA according to the recommended procedure

Results and dissection

MB dye spectrum

The spectrum of the MB dye was taken to fixed the maximum wavelength (λ_{max}) that will be used in subsequent measurements by taking 0.5 mL of the dye and adding 0.5 mL of 1M HCl, completing the volume with distilled water to 10 mL and taking the spectrum against the blank solution as shown in Figure (3). The highest absorption of MB dye is at a wavelength of 664 nm. and this has been fixed in subsequent experiments.

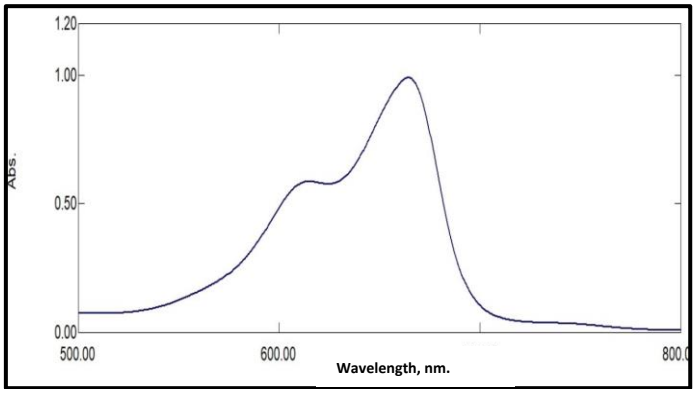

Figure 3: Absorption spectrum of MB dye

Dye volume effect

Different volumes of MB dye ranging from 0.1-1.7 mL were taken and the absorbance was measured at 664 nm against the blank solution, and the results are shown in Figure (4). It is clear that the linearity continues to 1.7 mL, with determination coefficient (R^2) of 0.9953. 0.6 mL of dye was chosen to give it an acceptable absorbance and that it is within the calibration curve.

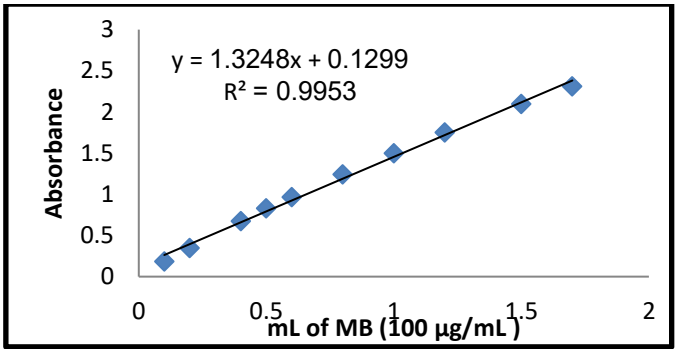

Figure 4: Calibration curve of MB dye

Selection of the oxidizing agent

The effect of various oxidizing agents on the bleaching the colour of MB dye was studied by preparing a number of oxidizing agents at a concentration of 1 x 10^{-3} mol/L. The oxidizing agents that were used are (N-bromosuccinimide, N-chloromosuccinimide, potassium periodate, and sodium periodate) by adding 1 ml of the oxidizing agent to 10 mL volumetric flask. contains 0.6 mL of dye and 0.5 mL of hydrochloric acid. The solutions are left for five minutes, then the volume is completed with distilled water to the mark, after which the absorbance is measured at a wavelength of 664 nm against the blank solution. Figure (5) show that the oxidizing agent N-bromosuccinimide gives the best bleaching process was therefore chosen for subsequent experiments.

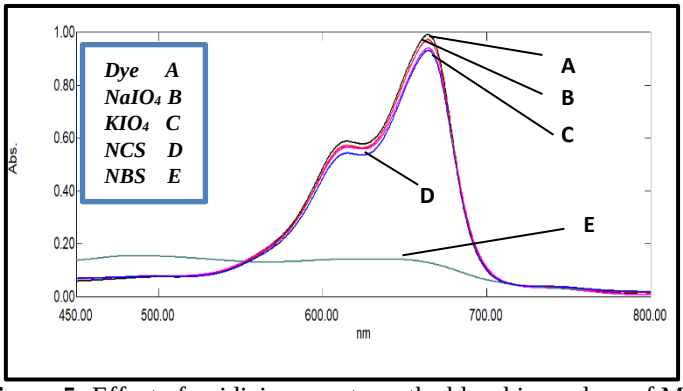

Figure 5: Effect of oxidizing agents on the bleaching colour of MB

Effect of the amount of oxidizing agent on the color dye.

The effect of the amount of N-bromosuccinimide (NBS) on bleaching colour of the dye in an acidic medium was studied by adding different volumes of the oxidizing agent ranging from 0.2-1.7mL to 0.6 mL of the dye and 0.5 mL of hydrochloric acid. The solutions were left for five minutes, then the volume was supplemented with distilled water to an extent mark then measured at the wavelength of 664 nm. against the blank solution, and the results are shown in Figure (6). It was inferred that the volume of 1 mL of the (NBS) oxidizing agent is the best to give it the best bleaching of the dye, so it was adopted by the subsequent experiments.

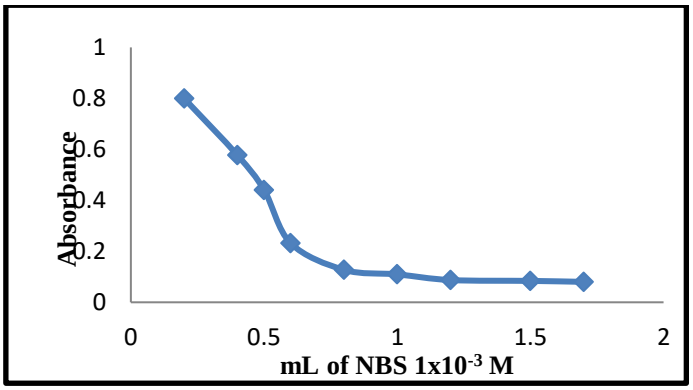

Figure 6: The optimal amount of NBS oxidant in bleaching colour

Acid type effect

The effect of various types of acids on the oxidation of NICA was studied by preparing a number of acids at a concentration of 1 M, Hydrochloric acid (HCl) was chosen to give it the highest absorbance value meaning more oxidation of NICA the result shown in Table (1).

Table 1: Choosing the appropriate acid for the oxidation process

Acid*	Absorbance	pH
HCl	0.7631	**1.91**
HNO_3	0.4842	**2.02**
H_2SO_4	0.1929	**2.03**
CH_3COOH	0.4544	**3.34**
H_3PO_4	0.2693	**2.32**

*0.5 mL of acid used.

Effect of the amount of acid

The effect of the amount of hydrochloric acid required to complete the niclosamide oxidation process has been studied, as shown in Table (2).

Table 2: Choosing the best volume of acid for the oxidation process

mLof 1M HCl	Absorbance	pH
0.25	0.7080	**2.17**
0.50	0.7681	**1.93**
0.75	0.7751	**1.90**
1.00	0.7845	**1.80**
1.25	0.7631	**1.76**

The optimal volume 1 mL of hydrochloric acid it gave the highest absorbance of the unreacted dye, so this volume of acid was adopted in subsequent experiments.

Oxidation time of NICA and dye bleaching

The time required for the oxidation of NICA was studied by adding 1 ml of N-bromosuccinimide with 1 ml of acid and 0.5mL NICA to volumetric flasks of 10 mL and left for different periods of time (2 -10) minutes, then 0.6 mL of MB dye was added, before diluted to the mark, left for different periods of time. From (2-20) minutes to bleach the dye and then measure the absorbance, the result shown in Table (3).

Table 3: Selection of oxidation time and bleaching dye time.

Standing time of oxidation	Standing time before dilution				
	2	5	10	**15**	20
2	0.4895	0.4559	0.4546	0.3959	0.3754
5	0.6458	0.6534	0.6695	0.6384	0.6256
10	0.7857	0.7890	0.7906	**0.8021**	0.7990

The best oxidation time is 10 minutes and the best time to bleach the colour of the MB dye is 15 minutes after adding the dye, so it was adopted in subsequent experiments.

Colour stability of the remaining MB dye

The colour stability of the residual dye was studied by taking two different concentrations of niclosamide and prepared according to the recommended procedure reading the absorbance at different times ranging from 2 to 60 minutes. The results are shown in Table (4).

Table 4: Colour stability of the remaining dye

Time (min.)	Absorbance of unreacted dye in presence 2µg/mL NICA	Absorbance of unreacted dye in presence 4µg/mL NICA
2	0.3728	0.6478
5	0.3736	0.6462
10	0.3745	0.6366
15	0.3736	0.6272
20	0.3697	0.6245
25	0.3651	0.6182
30	0.3645	0.6142

35	0.3604	0.6129
40	0.3588	0.6106
45	0.3558	0.6078
50	0.3551	0.6045
55	0.3544	0.6013
60	0.3539	0.5999

From the results listed in Table (4), it is clear that the dye is stable form 15 to 60 minutes.

The optimal condition gated from the all experiments above were listed in table (5).

Table 5: Inducted optimal conditions gated from the propos experiments

Parameters	Optimum conditions
λ_{max} (nm.)	664
Type, amount of acid used (1M)	HCl, 1mL
Type, amount of oxidant (1x 10^{-3}M)	NBS ,1 mL
Amount of MB dye (0.01%)	0.6 mL
Oxidation, Bleaching time (min.)	10 , 15
Solvent	Distilled water

Final absorption spectra

When adding 1mL of the oxidizing agent NBS (1x10^{-3}M) to the 0.5 mL of niclosamide NICA (100 µg / mL) under optimal conditions(Table 5) and after making the environment acidic by adding 1 mL of 1 M hydrochloric acid to a volumetric flask of 10 mL. and waiting for 10 minutes at room temperature, 0.6 mL of the dye MB (0.01%) is added, left for 15 minutes, and after completing the volume to the mark, the absorption spectrum of the remaining dye is measured against the blank solution. Figure (7) shows the final absorption spectrum of the dye alone against the blank solution and the absorption spectrum of the dye remaining against the blank solution.

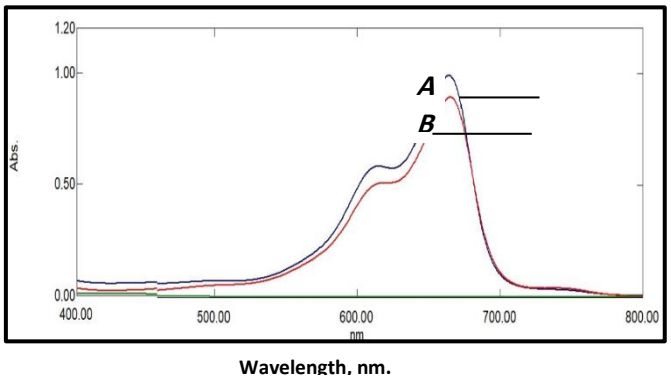

Figure 7: Absorption spectra of 50 µg/10 mL of NICA measured. (A): Dye versus blank solution (B): Remaining dye versus blank solution.

Accuracy and precision

Under the optimal conditions shown in the mentioned working method, the accuracy and precision of the method were calculated by taking two concentrations (3and 5 µg. mL^{-1}), the percentage of recovery, relative error and relative standard deviation were calculated as shown in Table (6). Which indicates that the method has good accuracy and precision.

Table 6: Accuracy and precision of the proposed method.

Conc. of niclosamide (µg/ml)	Recovery *%	RE %	RSD%	Average Recovery%
3	99.10	-0.90	1.93	99.47
5	99.85	-0.15	0.65	

*****Average of five determinations.

Application of the proposed method to pharmaceutical preparations:

The proposed method was applied for the determination of niclosamide compound in the pharmaceutical preparation (Yomesan Tablets), produced by the German Company Bayar Vital, by taking different volumes of the medicinal product solution (100 µg.mL^{-1}) to obtain concentrations 3 and 5 µg.mL^{-1}, and it was treated

according to the described method of work for standard solutions, and the obtained results are summarized in the Table (7).

Table 7: Application of the method to the pharmaceutical preparation.

Pharmaceutical preparation	µg NICA Present/ mL	µg NICA Measured/ mL	Recovery, %	Relative standard deviation*,%	Drug content mg measured	t_{exp}**[16]
Yomesan 500 mg/Tablet Germany Bayar vital	3	3.01	100.33	1.71	501.65	0.325
	5	5.16	103.20	0.62	516.00	0.571

*Average of five determinations.

** $t = (X - M).\sqrt{n}/S$, 2.132 t (tablet)

Application of the proposed method to veterinary preparation.

The proposed method was applied to determine the niclosamide compound in the veterinary product (Niclosam tablets), produced by the Iranian Zagros Pharmed Company, by taking different volumes of drug solutions 100 µg / mL to obtain concentrations of 3 and 5 µg. mL^{-1} and it was treated according to the described method of work for standard solutions, the obtained results are summarized in the Table (8).

Table 8: Application of the method to the veterinary preparation.

Veterinary preparation	µg NICA Present/ mL	µg NICA Measured/ mL	Recovery%	Relative standard deviation*,%	Drug content mg measured	t_{exp}
Niclosam 1250 mg /Tablet Iran Zagros Pharmed	3	2.90	96.66	1.77	1208.25	1.038
	5	5.03	100.60	1.58	1257.50	0.669

*Average of five determinations.

The results shown in Tables (7) and (8) prove the success of the method in estimating niclosamide in its pharmaceutical and veterinary preparations, as it gave a good percentage recovery for the active substance , a content value for niclosamide within the acceptable range, and the t_{exp} less than the tablet value too.

Standard addition method

To find out that the proposed method is characterized by accuracy and that it is free from interference of addition used in manufacturing formation, the standard addition method was used to estimate NICA in pharmaceutical and veterinary preparations. From drawing the relationship between concentration and absorbance for both veterinary and pharmaceutical preparations. The results shown in Figure (8, a and b) and Table (9).

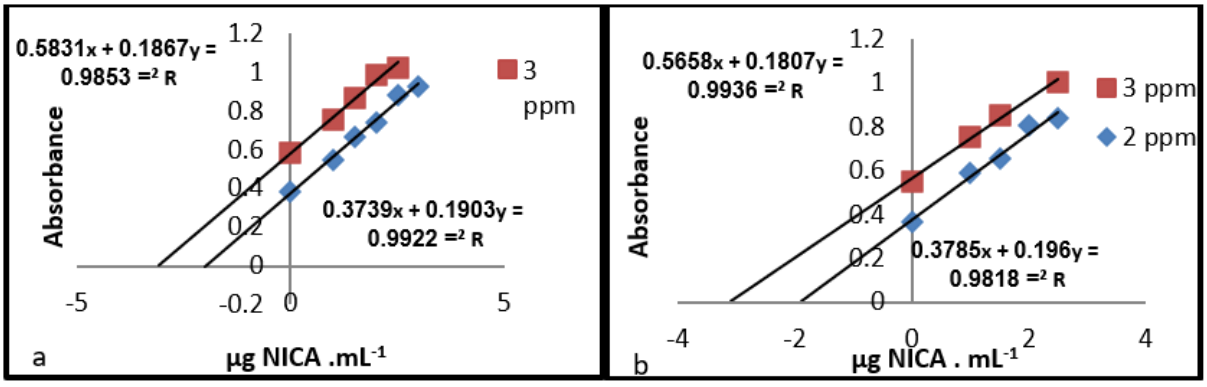

Figure 8: Plot of standard addition method estimating of NICA in tablets, (a) veterinary and (b) pharmaceutical preparations.

Table 9: Results of the standard addition method for NICA estimation.

Drug	NCIA TAKEN µg/ mL	NCIAfound µg / mL	Recovery %	Drug content(mg) measured
Niclosam 1250 mg/ Tablet Iran Zagros Pharmed	2	1.96	98.00	1225
	3	3.12	104.00	1300
Yomesan 500 mg/Tablet Germany Bayar vital	2	1.93	96.50	482.50
	3	3.13	104.33	521.65

Conclusions

Simple, delicate and sensitive spectrophotometric method was developed for the determination of NICA as pure , pharmaceutical and veterinary formulations. The method included adding an excess of the oxidizing agent NBS, followed by adding a specific volume of the MB dye, where the NICA is oxidized, and the remaining MB bleaching. The concentration of NICA was found by measuring the absorbance of the remaining dye, which is directly proportional to that of NICA. The method was successfully applied in the determination of NICA in pharmaceutical and veterinary preparations.

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