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Developing the analytical methods for the assay of anthelmintics (Albendazole) and anti-tuberculosis agents (Ethionamide) by utilizing two CT reagents - iodine and picric acid

Saurabh Savita
Institute of Pharmacy, PK University, Thanra - 473665, Madhya Pradesh, India
Corresponding author email: saurabhsavi93@gmail.com

Niyati Shrivastava
Institute of Pharmacy, PK University, Thanra - 473665, Madhya Pradesh, India

Sunil Kumar
Institute of Pharmacy, PK University, Thanra - 473665, Madhya Pradesh, India

Vinay Kumar Siroliya
Institute of Pharmacy, PK University, Thanra - 473665, Madhya Pradesh, India

Varsha Yadav
Institute of Pharmacy, PK University, Thanra - 473665, Madhya Pradesh, India

Prachi Gupta
Institute of Pharmacy, PK University, Thanra - 473665, Madhya Pradesh, India

***Abstract*---**Introduction - Rifampin, isoniazid, pyrazinamide, and ethambutol are antitubercular drugs that have been licenced by the FDA for the treatment of Mycobacterium tuberculosis infections. Antitubercular medicines are drugs that are used to treat tuberculosis. The term "anthelmintic" refers to a medicine used to treat parasitic worm infestations in animals. This comprises both flat and round worms, such as flukes (trematodes) and tapeworms (cestodes) (nematodes). Aim of this study - The primary aim of this research is to develop and validate simple, fast, reliable, robustness and ruggedness analytical procedures for anthelmintics (ALB) and antitubercular (ETH) medicines. Material and Methods - Two CT reagents, iodine and picric acid, were used to produce two simple and somewhat sensitive Albendazole (ALB) and Ethionamide (ETN) techniques. The CT complex with the placebo blank had almost the

same absorbance as the reagent blank, indicating that there was no interference by using Systronics model 166 digital spectrophotometer tools. Organic solvents were employed to dissolve all of the chemicals, which were of analytical reagent grade. Data analysis - The data has been analysed by created a synthesised mixture by combining 5 mg ETN with 5 mg placebo blank and homogenising the mixture. The placebo blank and synthetic mixture solutions were prepared from the reaction as the tablets, and a suitable aliquot was subjected to analysis by Picric acid method (n=5) after appropriate dilution by Iodine method. To assess the ruggedness, 3 distinct analysts used the same cuvette, and a single analyst used three different cuvettes. Conclusion - It is concluded that the proposed methods were both robust and rugged. The results of the recovery study concluded that the common tablet excipients did not interfere in the assay procedures.

***Keywords*---**anthelmintics, anti- tuberculosis, albendazole, ethionamide, iodine, picric acid.

Introduction

Anthelmintics

Anthelmintics are anthelminthic medications that kill parasitic worms. Helminths, also known as flukes, roundworms, and tapeworms, are parasitic worms that look like worms. Other anthelmintics are poorly absorbed via the gut, exposing the parasite to considerably higher anthelmintic concentrations than the host [1-3]. The term "anthelmintic" refers to a medicine used to treat parasitic worm infestations in animals. This comprises both flat and round worms, such as flukes (trematodes) and tapeworms (cestodes) (nematodes) [4, 5]. Parasites are extremely important in both human and veterinary tropical medicine.

Types [6]

- Benzimidazoles:
  - ✓ Albendazole
  - ✓ Mebendazole
  - ✓ Thiabendazole
  - ✓ Fenbendazole
  - ✓ Triclabendazole
  - ✓ Flubendazole
- Abamectin
- Diethylcarbamazine
- Pyrantel pamoate
- Levamisole
- Salicylanilide
  - ✓ Niclosamide
  - ✓ Oxyclozanide
- Nitazoxanide

- Oxamniquine
- Praziquantel
- Octadepsipeptides (e.g., Emodepside)
- Monepantel (aminoacetonitrile class)
- Spiroindoles (e.g., derquantel)
- Artemisinin

Antituberculosis agents?

Antituberculosis agents are medications which are used to treat tuberculosis, a disease caused by bacterial Mycobacterium tuberculosis. This infection primarily affects the lungs, but it can damage a variety of other organ systems as well. Several medication classes have activity against Mycobacterium TB, each with a different mode of action. Chemotherapy for tuberculosis entails administering two to four medications at the same time. Because these treatments work in diverse ways, they attack the organism in different ways, and utilising only a few types of drugs prevents drug-resistant Mycobacterium strains from forming [7-12].

Types [7]

- Rifampicin (RIF)
- Isoniazid (INH)
- Ethionamide (ETN)
- D-cycloserine (DCS) etc.

Aim of the study

The main aim of this study is to discuss the Developing the Analytical Methods for The Assay of Anthelmintics (Albendazole) And Anti- Tuberculosis Agents (Ethionamide) By Utilizing Two Ct Reagents - Iodine and Picric Acid.

Experimental Setup

Apparatus

The absorbance readings were recorded using a Systronics model 166 digital spectrophotometer (Systronics Ltd., Ahmedabad, Gujarat, India) with 1-cm path length matched quartz cells.

Reagents and chemicals

Organic solvents were employed to dissolve all of the chemicals, which were of analytical reagent grade. In dichloromethane and chloroform, solutions of 0.3 percent iodine (Merck Ltd., Mumbai, India) and 0.05 percent picric acid (S.D. Fine Chem., Mumbai, India) were produced separately.

Standard ALB solution

Dissolving 40 mg of pure drug in dichloromethane and diluting to volume with the same solvent in a 100 mL calibrated flask yielded a stock standard solution containing 400 µg mL^{-1} ALB, which was employed in the iodine procedure. A 100-µg mL^{-1} ALB solution was prepared by dissolving 10 mg of pure drug in chloroform and diluting to mark in a 100 mL calibrated flask with much the same solvent, and a working concentration of 60 µg mL^{-1} was produced by diluting with chloroform.

Standard ETN solution (50 & 100 µg mL^{-1}) for ethionamide

A stock standard solution containing 100 µg mL^{-1} ETN was made by dissolving 10 mg of pure drug in chloroform and diluting to volume with the same solvent in a 100 mL volumetric flask, which was then utilized in the Picric acid procedure. For the Iodine technique, this has been diluted to a workable concentration of 50 µg mL^{-1} by diluting with chloroform.

Assay Procedures

Assay Procedures for Albendazole (ALB)
Procedure for bulk drug

- **Iodine method**: Aliquots of standard ALB solution (400 µg mL^{-1}) were accurately poured into 5 mL standard flasks using a micro burette, and the total volume was corrected to 3.0 mL with dichloromethane. Each flask received one mL of 0.3 percent iodine, which was diluted to volume with the same solvent and thoroughly mixed. After 5 minutes, the absorbance of each concentration was taken at 380 nm against a reagent blank.
- ***Picric acid method:*** A micro burette has been used to accurately add aliquots of 0.2, 0.5, 1.0, 2.0, 3.0, and 3.5 mL of standard ALB solution (60 µg mL^{-1}) to 5 mL calibrated flasks, and the total volume was adjusted to 3.5 mL using chloroform. Each flask received one mL of 0.05 percent picric acid, which was diluted to volume with chloroform and thoroughly mixed. The absorbance of each solution was measured at 415 nm against a reagent blank after 5 minutes. The concentration of the unknown was estimated from the corresponding regression equation derived using Beers law data, and the standard graph was produced by graphing the absorbance vs drug concentration.

Procedure for formulations

In a 50 mL calibrated flask, a carefully weighed quantity (tablet powder or suspension) equal to 20 mg of ALB was transferred and dissolved in 30 mL of dichloromethane. The contents were thoroughly shaken for 15-20 minutes, then diluted to the desired concentration with dichloromethane, mixed thoroughly, and filtered using a Whatman No. 42 filter paper. The first 5 mL of the filtrate was discarded, and an appropriate aliquot (400 µg mL^{-1}) of the filtrate was subjected to iodine technique analysis. A properly weighed quantity of tablet powder or suspension equivalent to 5 mg of ALB was put into a 50 mL calibrated flask and

agitated with 30 mL chloroform for the picric acid technique; its extraction was made as stated previously. Before being tested, the extract was diluted to a working concentration of 60 μg mL^{-1} by taking a sufficient aliquot.

Procedure for selectivity study

Two placebo blanks were made in dichloromethane and chloroform, respectively, and evaluated using the two procedures. Following the stages outlined under 'protocol for formulations,' extracts of the synthetic combination were made independently for the iodine and picric acid methods, and examined to use the general procedures.

Assay Procedures for Ethionamide (ETN)
Procedure for bulk drug

- ***Iodine method:*** A micro burette had been used to accurately transfer 0.1, 0.25, 0.5, 1.0, 1.5, and 2.0 mL of 50 μg mL^{-1} standard ETN solution into 5 mL calibrated flasks, and the total volume was adjusted to 2 mL with choloroform. Each flask received one mL of 0.2 percent iodine, which was diluted to the mark with choloroform and thoroughly mixed. After 5 minutes, the absorbance of each solution was measured at 400 nm against a reagent blank.
- ***Picric acid method:*** Using a micro burette, aliquots of 100 μg mL^{-1} standard ETN solution of 0.1, 0.25, 0.5, 1.0, 1.5, 2.0, and 2.25 mL were precisely added to 5 mL standard flasks, and the total volume was made to 2.25 mL using choloroform. Each flask received one mL of 0.1 percent picric acid, which was diluted to the mark with choloroform and thoroughly mixed. The absorbance of each solution was measured at 420 nm against the reagent blank after 5 minutes.

Procedure for tablets

A tablet powder adding 5 mg of ETN was carefully weighed and put to a 50 mL standard flask, where it was agitated for 15 minutes with 20 mL of choloroform. The volume was completed with chloroform and then combined. A Whatman No. 42 filter paper was used to filter it. For the iodine technique, the filtrate was diluted to obtain 50 μg mL^{-1} ETN. Following the prescribed protocol, one mL of tablet extract was evaluated in five repetitions.

Procedure for selectivity study

The placebo blank used was identical to the one described. 5 mg ETN was mixed with 5 mg placebo blank to make a synthetic combination, which was then homogenized. The placebo blank and synthetic mixture solutions were made using the same manner as the tablets, and a sufficient aliquot was subjected to statistical analysis by Picric acid method (n=5) after appropriate dilution by Iodine technique.

Data Analysis on Anthelmintics (Albendazole) Agent

Absorption spectra

When iodine or picric acid reacts with ALB, it produces a strong wine red or yellow coloured product. Colored products' absorption spectra were measured at 360-480 nm in comparison to reagent blanks. For ALB-I_2 and ALB-PA, the coloured charge-transfer compounds had maximum absorbance at 380 nm and 415 nm, respectively.

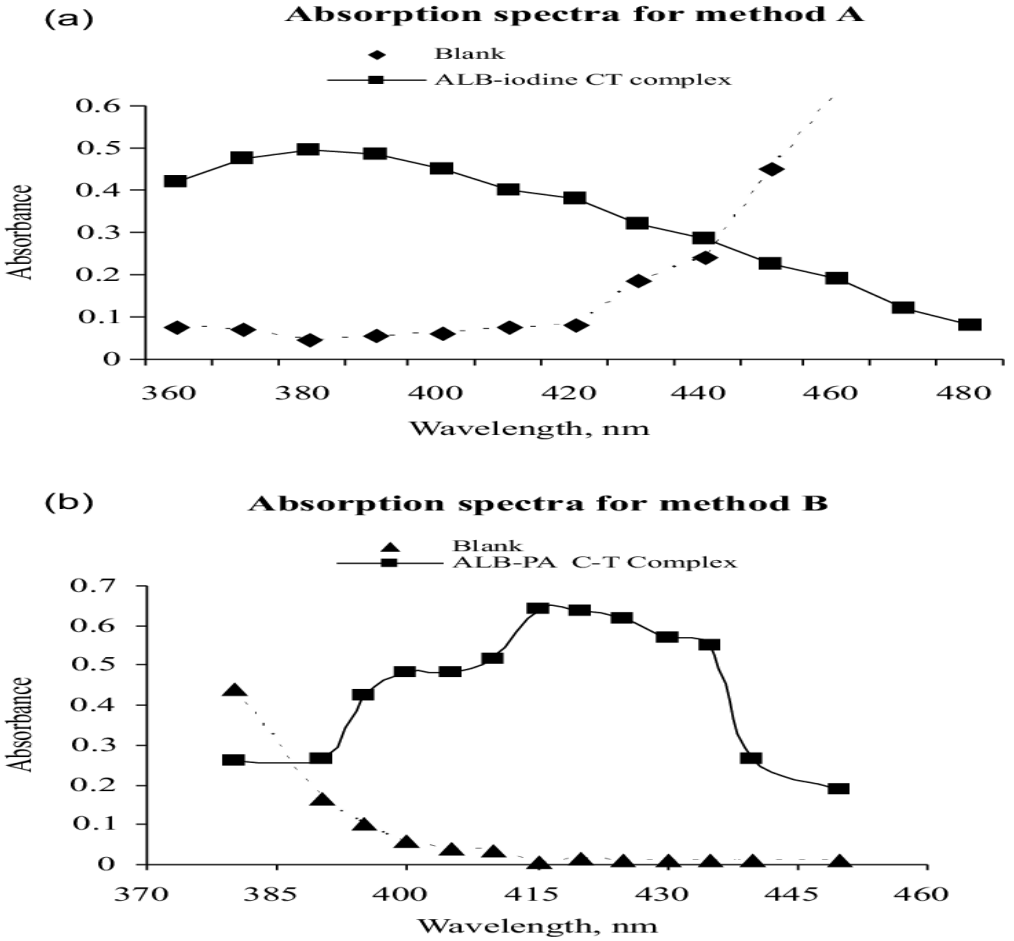

Figure 1. Absorption spectra (a) ALB-iodine CT complex (-▪-) [120 µg mL^{-1}ALB] against blank solution (-▪-) and (b) ALB- picric acid CT complex(-▪-) [30 µg mL^{-1} ALB] against blank solution (-▪-)

Optimization of experimental variables
Effect of reagent concentration

This was investigated by varying the amount of iodine or picric acid added to a constant concentration of ALB. 1 mL of reagent solution were considered

sufficient in each case for the production of coloured species with maximum and repeatable colour intensity. (Fig. 2).

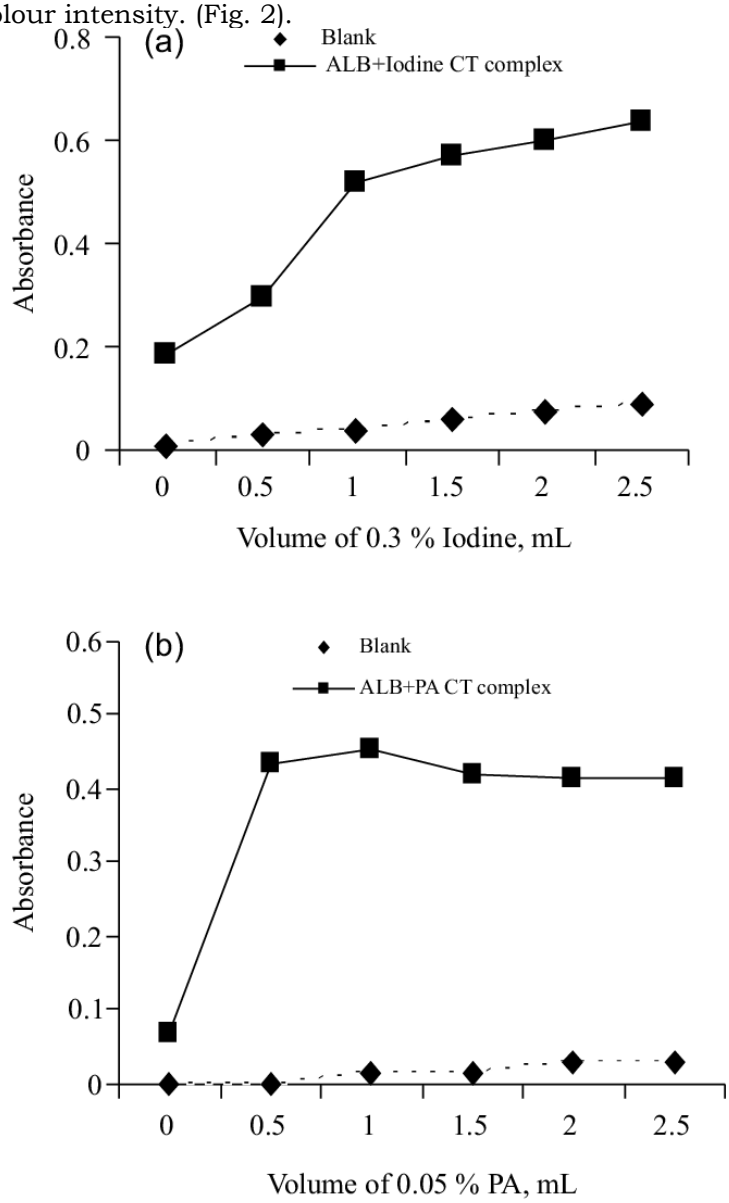

Figure 2. Impact of reagent concentration (a) ALB-iodine CT complex (-▪-) [120 µg mL^{-1} ALB] and blank solution (-▪-); (b) ALB- picric acid CTcomplex (-▪-) [30 µg mL^{-1} ALB] and blank solution (-▪-)

Effect of solvent

The reaction of ALB with iodine or picric acid was carried out in a variety of solvents, including dichloromethane, chloroform, acetonitrile, methanol, 1,2-dichloroethane, 1,4-dioxane, and benzene, in order to determine the best solvent for charge-transfer complex production. Dichloromethane was discovered to be

ideal for the iodine method, whereas chloroform was shown to be ideal for the picric acid method (Fig. 3). Similarly, the influence of the diluting solvent was investigated for both procedures, with the results indicating that dichloromethane and chloroform were the best diluting solvents for the iodine and picric acid techniques, correspondingly.

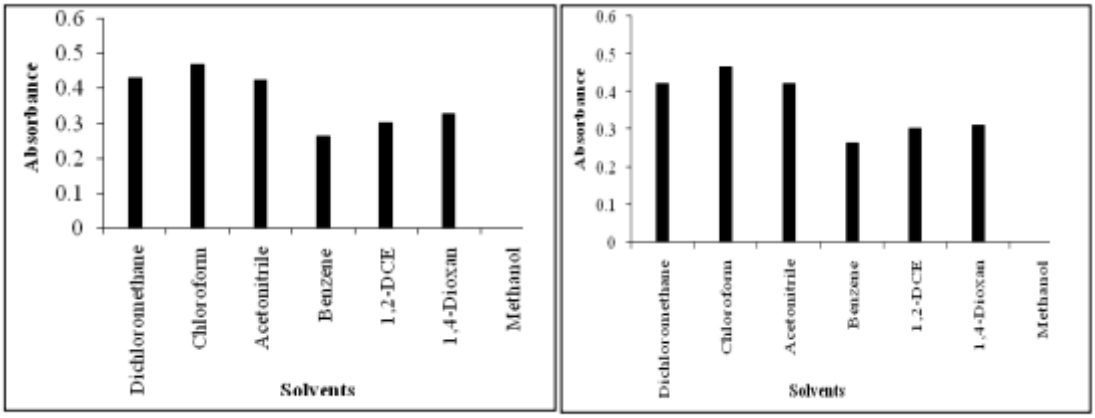

Figure 3. Effect of different solvent (a) ALB-iodine CT complex (-▪-) [120 µg mL^{-1} ALB]; (b) ALB-picric acid CT complex (-▪-) [30 µg mL^{-1} ALB]

Validation of Method

Analytical parameters

The least squares method was used to create the linear regression equations, and Table 1 shows the Beer's law range, molar absorptivity, correlation coefficient, and confidence limits for slope and intercept for both methods. Table 1 also shows how the detection limit (LOD) and limit of quantification (LOQ) were derived using ICH recommendations.

Table 1
(ALB) Parameters of Sensitivity and regression

Parameter	Iodine method	Picric acid method
λ_{max}, nm	380	415
Color stability, min.	30	120
Linear range, µg mL^{-1}	8.0-240	2.4–42
Molar absorptivity (ε), L mol^{-1} cm^{-1}	1.17×10^{3}	5.22×10^{3}
Sandell sensitivity*, µg cm^{-2}	0.2273	0.0509
Limit of detection (LOD), µg mL^{-1}	0.69	0.10
Limit of quantification (LOQ), µg mL^{-1}	2.08	0.30
Regression equation, Y**		
Intercept (a)	0.0515	0.0137
Slope (b)	0.0036	0.0193
Standard deviation of a (S_a)	1.14×10^{-3}	0.98×10^{-3}

Standard deviation of b (S_b)	5.0×10^{-4}	2.24×10^{-3}
Regression coefficient (r)	0.9991	0.9984

*The weight in μg mL^{-1} of solution corresponds to an absorbance of A = 0.001 measured in a cuvette with a cross-sectional area of 1 cm^2 and a length of 1 cm. *Y=a+bX, where Y is the absorbance, X is the concentration in μg mL^{-1}, and a and b are the intercept and slope, respectively.

Robustness and ruggedness

Two crucial experimental variables, reagent amount and reaction time, were gently adjusted to determine the robustness of the suggested procedures, and the influence of these adjustments on the absorbance of the CT complex was investigated. As part of the ruggedness investigation, three analysts worked on the same instrument and used the same three cuvettes. The recommended approaches were found to be both robust and ruggedness based on the percent RSD values reported in Table 2.

Table 2
Intermediate Precision (%RSD) method of robustness and ruggedness

Method	ALB taken (μg mL^{-1})	Method robustness			Method ruggedness	
		Parameters altered				
		Volume of iodine %RSD (n=3)	Volume of picric acid %RSD (n=3)	Reaction time %RSD (n=3)	Inter-analysts, % RSD (n=3)	Inter-cuvettes, %RSD (n=3)
Iodine method	20.0	1.26	-	1.61	1.52	1.63
	80.0	1.78	-	1.24	2.03	1.84
	200.0	2.09	-	2.13	3.12	2.98
Picric acid method	12.0	-	1.27	1.74	1.12	1.36
	24.0	-	0.93	1.18	0.96	1.21
	36.0	-	1.85	1.02	0.89	2.35

In both methods, volumes of reagent were 1 and 1±0.1 mL and reaction times were 5 and 5±1 min.

Selectivity

The placebo blanks did not absorb significantly when evaluated using the proposed procedures. For the iodine and picric acid methods, the % recoveries of ALB from synthetic mixed solution were 98.36±2.3 and 101.2±2.8, correspondingly. This validated the procedures' selectivity in the presence of common tablet adjuvants.

Application to formulations

The proposed methods used to determine ALB concentrations in tablets and suspension. The active content of the same batch of tablets was determined using the approved method, and the results were compared using the student's t-test

for accuracy and the F-test for precision. Table 3 shows that the estimated t- and F-values for four degrees of freedom did not surpass the tabulated values of 2.78 and 6.39, respectively, at the 95 percent confidence level. This demonstrated that both the proposed and official procedures are equally accurate and exact.

Table 3
Analytical results of formulations by the proposed methods of ALB

Formulation analyzed	Label claim, mg/tablet	Found* (Percent of label claim±SD)		
		Proposed methods		
		Reference method	Iodine method	Picric acid method
Alworm-400	400	101.3±1.02	100.1±0.83 t = 2.01 F = 1.51	102.7±2.04 t = 1.37 F = 4.0
Bandy-400	400	99.80±1.17	99.50±1.13 t = 0.41 F = 1.07	100.3±0.47 t = 0.89 F = 6.2
Zentel suspension	400	100.8±0.91	99.80±0.44 t = 2.23 F = 4.93	101.4±1.65 t = 0.71 F = 3.29

* The mean of five measurements.

For four df, the tabulated t-value is 2.77 and the tabulated F-value is 6.39 at a 95% confidence level.

Recovery studies

Pure ALB was spiked into pre-analyzed tablet powder and Zentel suspension at three distinct concentration levels (50, 100, and 150 percent of the large set in the formulation), and the total was calculated using the recommended techniques.

Data Analysis on Anti-Tuberclosis (Ethionamide) Agent

Absorption spectra

At 370-470 nm, the absorption spectrum of coloured product generated in either process was measured against a reagent blank. The ETN-I_2 and ETN-PA complexes produced colourful products with maximal absorbance at 400 and 420 nm, correspondingly (Fig. 4).

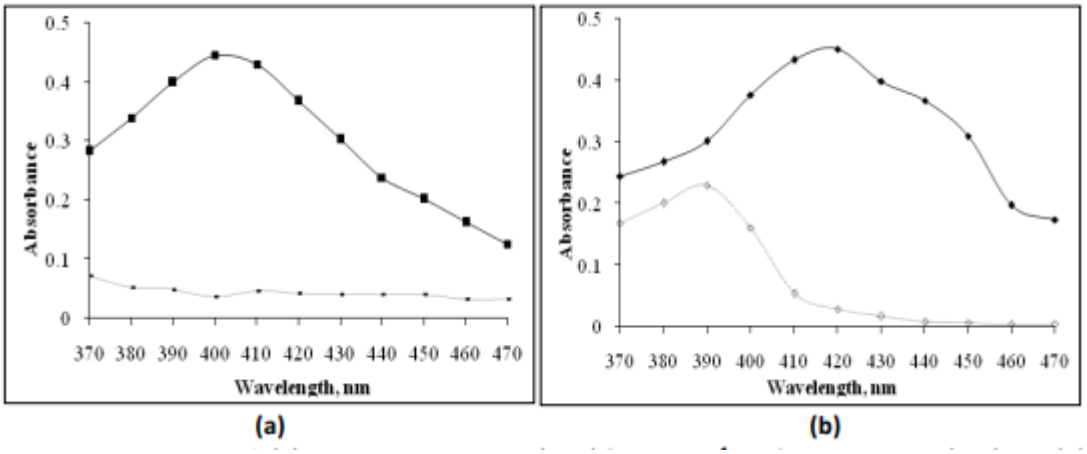

Figure 4. Absorption spectra (a) ETN-iodine complex (-■-) [10 µg mL^{-1} ETN] against blank (-■-) (b) ETN-picric acid complex (-■-) [20µg mL^{-1} ETN] against blank (-■-)

Optimization of reaction conditions
Choice of solvent

Different solvents have been used as reaction medium, including dichloromethane, chloroform, acetonitrile, benzene, 1,2-dichloroethene, 1,4-dioxan, and methanol, and the reaction of ETN with iodine or picric acid was observed (Fig. 5). Chloroform was the best choice for both the reaction medium and the diluent in both techniques.

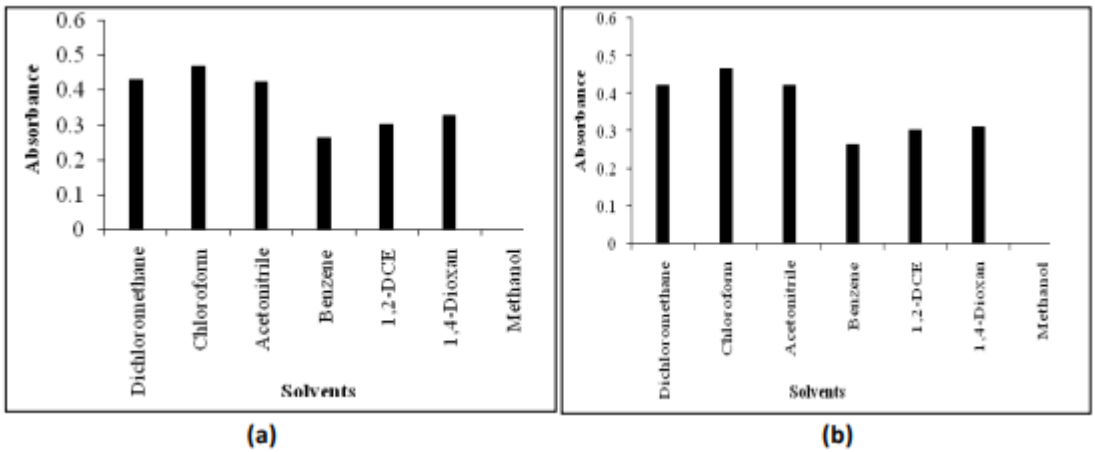

Figure 5. Impact of different solvents (a) ETN-iodine complex (-■-) [10 µg mL^{-1} ETN] (b) ETN-picric acid complex (-■-) [20 µg mL^{-1} ETN]

Effect of reagent concentration

The influence of acceptor concentration on the formation of complexes was studied. In both approaches, one mL of acceptor solution was found to be

sufficient. Higher blank absorbance was observed in both approaches, despite the fact that bigger volumes seemed to have no effect on complex formation (Figure 6).

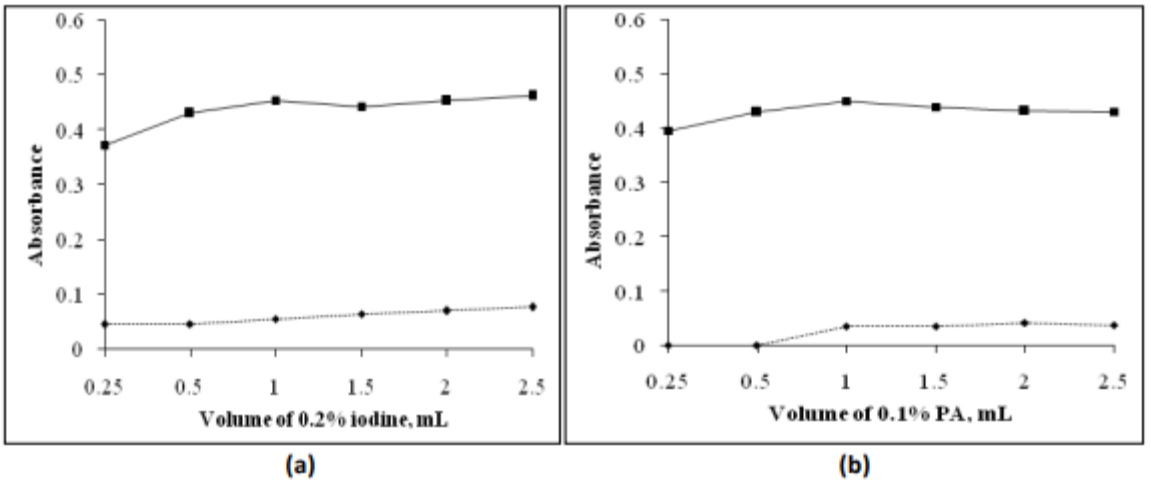

Figure 6. Impact of volume (a) 0.2% iodine in iodine method (10 µg mL^{-1}ETN) (b) 0.1% picric acid in picric acid method (20 µg mL^{-1} ETN)

Validation of Method

Analytical parameters

Absorbance and absorbance were shown to have a linear relationship. at λ_{max} and ETN concentrations in the ranges listed in Table 4 Table 4 shows the slopes, intercepts, and correlation coefficients of the linear plots (Figure 7). Table 4 also lists the optical features of both methods, such as Beer's law limitations, molar absorptivity, Sandell sensitivity values, limits of detection (LOD), and quantification (LOQ).

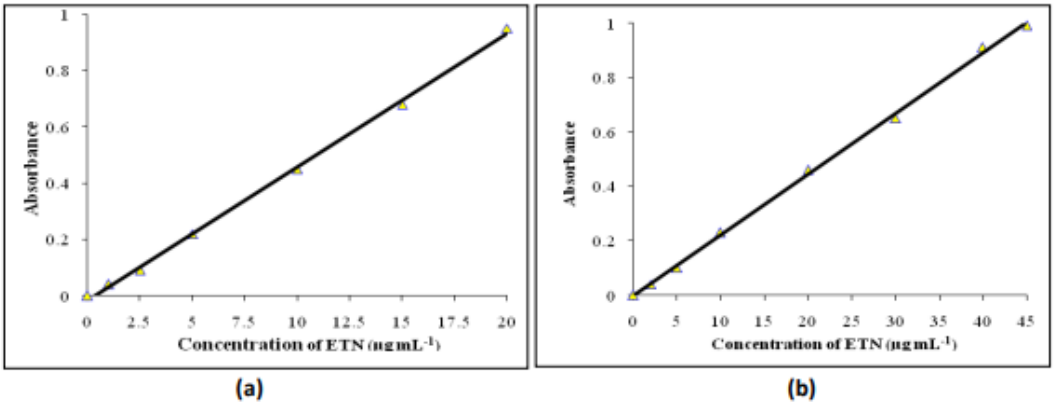

Figure 7. Calibration curves: (a) iodine method and (b) picric acid method

Table 4
(ETN) Parameters of Sensitivity and regression

Parameter	Iodine method	Picric acid method
λmax, nm	400	420
Colour stability, h	1 h	4 h
Linear range, µg mL-1	1–20	2–45
Molar absorptivity (ε), L mol-1 cm-1	7.15×103	3.62×103
Sandell sensitivity*, µg cm-2	0.0495	0.0459
Limit of detection (LOD), µg mL-1	0.24	0.96
Limit of quantification (LOQ), µg mL-1	0.73	2.92
Regression equation, Y**		
Intercept (a)	-0.0213	-0.0018
Slope (b)	0.0478	0.0223
Standard deviation of a (Sa)	2.28×10-4	1.2×10-5
Standard deviation of b (Sb)	1.72×10-4	1.41×10-4
Regression coefficient (r)	0.9994	0.9993

*The weight in µg mL^{-1} of solution corresponds to an absorbance of A = 0.001 measured in a cuvette with a cross-sectional area of 1 cm^2 and a length of 1 cm. *Y=a+bX, where Y is the absorbance, X is the concentration in µg mL^{-1}, and a and b are the intercept and slope, respectively.

Robustness and ruggedness

Two different experimental variables, reagent volume and contact time, were gently modified as part of the robustness investigation, and these modifications had very little impact on the assay results. Three distinct analysts used the same cuvette to measure toughness, whereas one analyst used three different cuvettes to determine ruggedness. Based on the percent RSD values in Table 5, it can be stated that the proposed procedures are both robust and ruggedness.

Table 5
Intermediate Precision (%RSD) method of robustness and ruggedness (ETN)

Method	ETN taken (µg mL-1)	Method robustness			Method ruggedness	
		Parameters altered				
		Volume of iodine, (n=3)	Volume of PA, (n=3)	Reaction time (n=3)	Inter-analysts, % RSD (n=3)	Inter-cuvettes, %RSD (n=3)
Iodine method	5.0	2.05	-	1.71	1.54	1.61
	10.0	1.13	-	1.54	1.23	1.82
	15.0	2.16	-	2.03	2.85	2.08
Picric acid method	10.0	-	0.95	1.64	1.02	1.32
	20.0	-	1.23	1.88	0.99	1.29
	30.0	-	1.35	1.07	1.49	2.15

The reagent quantities were 1 ±0.1 mL, respectively, and the reaction durations were 5 ± 51 minutes in both procedures.

Selectivity study

The CT complex's absorbance with the placebo blank was nearly comparable to the reagent blank's absorbance, indicating no interference. The % recoveries of ETN from the synthetic mixed solution for the iodine and picric acid methods were 97.95±0.92 and 101.5±1.54, correspondingly. This demonstrates the approaches' selectivity in the presence of tablet excipients.

Application to tablets

The proposed methods used to determine ETN in tablet form. The resulting data were statistically compared to those obtained using the approved procedure. The statistical figures in Table 6 show that there is no difference in accuracy and precision between the proposed approaches and the official method.

Table 6
Analytical results of formulations by the proposed methods of ETN

Formulation analyzed	Label claim, mg/tablet	Found* (Percent of label claim±SD)		
		Proposed methods		
		Reference method	Iodine method	Picric acid method
Ethide-250	250	98.9±1.08	99.45±1.02 t = 0.83 F = 1.12	99.12±1.32 t = 0.29 F = 1.49
Mycotuf-250	250	101.3±0.75	102.1±1.31 t = 1.18 F = 3.05	101.9±0.96 t = 1.10 F = 1.64

* The average of five measurements.
At the 95 percent confidence level, the calculated t-value is 2.77. The calculated F-value is 6.39 at a 95% confidence level.

Conclusion

The goal of this study was to create and validate simple, fast, reliable, robustness and ruggedness analytical procedures for anthelmintics and antitubercular medicines. Two CT reagents, iodine and picric acid, were employed to produce two simple and moderately sensitive techniques. Bromophenol blue and bromothymol blue are two sulphonthalein dyes used in ion-pair processes. In dichloromethane or chloroform medium, the generated CT complexes between ALB and iodine (iodine technique) or picric acid (picric acid method) were measured directly.

Findings of the study

The % recoveries of ETN from the synthetic mixed solution for the iodine and picric acid methods were 97.950.92 and 101.51.54, respectively. This demonstrates the approaches' selectivity in the presence of tablet excipients. It is concluded that the proposed methods were both robust and rugged. The results of the recovery study concluded that the common tablet excipients did not interfere in the assay procedures. It is concluded that the percent recoveries of

ETN from the synthetic mixture solution were calculated to be 97.950.92 for the iodine method and 101.51.54 for the picric acid method, respectively. This reflects the methods' selectivity in the presence of tablet excipients.

Future scope

In future it is hoped that new anthelmintics with novel modes of action will solve the problem of anthelmintic resistance. More focused approaches to Antihelmintic drug development are also ongoing, providing the fundamental data with the goal of not targeting peptidergic signalling pathways that resulted in a marketable drug. The future holds the promise that ALB and ETN agents may also directly contribute to drug discovery, and such efforts are critical in light of the growing threat of anthelmintic resistance to livestock and humans.

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