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HPLC, Densitometric and Visible-Spectrophotometric Determination of Triclabendazole and Ivermectin

Sawsan A. Abdel Razeq[1*], Asmaa O. El Demerdash[1] and Hoda F. El Sanabary[2]

[1]Department of Analytical Chemistry, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo, Egypt.
[2]National Organization for Drug Control and Research (NODCAR), Giza, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. Author SAAR designed the study and managed the analyses of the study. Author AOED performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author HFES managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Two methods were developed for simultaneous determination of triclabendazole and ivermectin, in addition to a Vis-spectrophotometric method for the analysis of ivermectin only. The first one was HPLC method in which efficient separation of two drugs was achieved on a C_{18} column with isocratic elution and a mobile phase composed of acetonitrile-methanol-0.005M KH_2PO_4 in a ratio of (60:30:10, v/v/v), pH 6. The linearity range was found to be 2.5-50 µg mL^{-1} for triclabendazole and 1-20 µg mL^{-1} for ivermectin with mean accuracy of 100.11% ± 0.99 and 100.08% ± 0.53, respectively. The second method was a densitometric evaluation of thin-layer chromatograms of the two drugs using a mobile phase of chloroform-acetone (8: 2, v/v). The plates were visualized under UV lamp at 254 nm where spots appeared at R_f 0.79 and 0.33 for triclabendazole and ivermectin, respectively. The chromatograms of the two drugs were measured densitometrically at

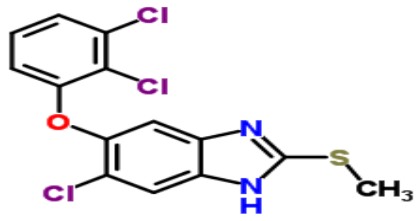
**Corresponding author: E-mail: razeqhegab@yahoo.com;*

306 nm in the range 2.5-25.0 µg/spot for triclabendazole and at 245 nm in the range of 0.5-5.0 µg/spot for ivermectin with mean accuracy of 100.38% ± 0.52 and 100.05% ± 0.41, respectively. The Vis-spectrophotometric method was applied for the determination of ivermectin depending on its reducing character on tetrazolium red to give highly colored red product absorbing maximally at 485 nm in the range of 10-200 µg mL^{-1} with mean accuracy of 100.61% ± 0.43. The first two methods provided selective recovery of triclabendazole and ivermectin (100.53% ± 0.97 and 100.03% ± 0.82, 99.94% ± 1.60 and 98.77% ± 0.72; respectively). While the third Vis-spectrophotometric method provided selective recovery of ivermectin (100.86% ± 0.93) in presence of triclabendazole. Successful application of the methods for analyzing triclabendazole and ivermectin in their pharmaceutical formulations was obtained. The validity of the method was evaluated in terms of linear regression analysis, precision, accuracy and selectivity.

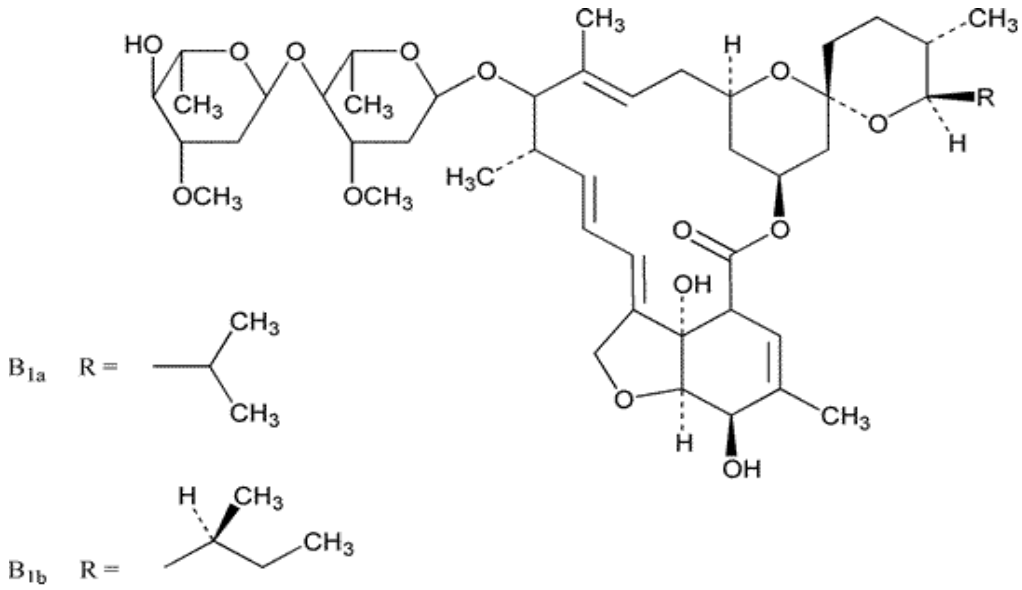
Keywords: HPLC; densitometry; Vis-spectrophotometric; tetrazolium red; triclabendazole; ivermectin.

1. INTRODUCTION

Triclabendazole,5-Chloro-6-(2,3-dichlorophenoxy)-2-(methylthio)-1H-benzimidazole [1], is a benzimidazole anthelmintic drug used in the treatment of veterinary and human fasciolasis and under investigation for the treatment of human paragonimiasis [2]. Ivermectin is a semisynthetic derivative of abamectin, which is one of the avermectins, a group of macrocyclic lactones produced by Streptomyces avermitilis, and consists of a mixture of not less than 80% component B1a (5-O-demethyl-22, 23-dihydroavermectin A1a) and not more than 20% component B1b (5-O-demethyl-25-de (1-methylpropyl)-25-(1-methylethyl)-22, 23-dihydroavermectin A1a) [1,2]. It has a microfilaricidal action in onchocerciasis and lymphatic filariasis and it is active in some other worm infections and used in the treatment of strongyloidia [2].



Triclabendazole



Ivermectin component H_2B_{1b} and component H_2B_{1a}

The literature comprises several analytical methods for the determination of triclabendazole and ivermectin in their pharmaceutical formulations and in the presence of their metabolites and impurities as well in environment, food, milk, biological fluids and tissues. HPLC is the most widely used tool for the analysis of both drugs [3-14]. Other reported methods include spectrophotometric method [15] and GC [16] for triclabendazole; while densitometric [17], GC [18], and capillary electrophoresis [19] and liquid phase microextraction methods coupled with LC [20, 21] were used for ivermectin analysis. Only two analytical methods have been described in literature for the simultaneous determination triclabendazole and ivermectin in binary mixture namely, RP-HPLC [22] and chemi- luminescent immunoassays [23]. The aim of the present study is to develop sensitive, selective and accurate HPLC and densitometric methods for the simultaneous determination of triclabendazole and ivermectin in their pharmaceutical formulations and a Vis-spectrophotometric method for ivermectin analysis.

2. EXPERIMENTAL

2.1 Instruments

- HPLC (Agilent 1100 series, Waldbronn, Germany) consists of an Agilent pump, equipped with a variable wavelength detector and a 20 µL volume injection loop, and Nucleosil column SC-04 120-5-C_{18} (125 mm × 4 mm).
- Densitometer-Dual Wave Length Flying Spot CS-9301 (Shimadzu, Tokyo, Japan).
- UV-Vis spectrophotometer (Shimadzu 2400, Japan).
- Thin layer chromatographic plates pre-coated with silica gel 60 F_{254}, 10x20 cm (Merck, Germany).
- Micro syringe 10 µL capacity (Hamilton, Germany).

2.2 Materials and Reagents

- Pure triclabendazole, B. N. 20080812, was kindly supplied by Pharaonia Pharmaceutical with purity of 99.5% as referred by the supplier.
- Pure ivermectin, B.N. 120070108, was kindly supplied by Norbrook Laboratories Ltd; 97.6% purity as referred by the supplier.
- Trimec Sheep – oral suspension; B.N. S111220, labeled to contain 50 mg of triclabendazole and one mg of ivermectin per mL, the product of Pharma Sweed, Cairo, Egypt; was purchased from a local market.
- Triclazole – oral suspension; B.N. 805646, labeled to contain 100 mg o triclabendazole per mL, the product of EVA Pharma for Pharmaceutical and Medical Appliance, Egypt; was purchased from a local market.
- Garlec – injection; B.N. 212002, labeled to contain 10 mg of ivermectin per mL, the product of Vetgreen Co. produced by Alex. Co. for Pharmaceutical and Chemical Industries packing by Arab Caps, Egypt; was purchased from a local market.
- Acetonitrile and Methanol, HPLC grade (Scharlau chemie, Spain).
- Absolute Ethanol and Methanol, (Sigma Aldrich, Germany).
- Acetone, Chloroform and Dichloromethane, (BDH Chemicals ltd, England).
- Potassium dihydrogen orthophosphate, (Merck, Germany); 0.005M aqueous solution.
- NaOH, (Nakamaruko Kawasaki, Japan); 0.2 M alcoholic solution, prepared by dissolving 0.8 g in minimum amount of water and completing volume to 100mL with ethanol [24].
- 2,3,5-Triphenyl tetrazolium chloride (tetrazolium red); (Reidel, Germany); 0.2% and $1x10^{-3}$ M alcoholic solution. The later solution was prepared by dissolving 33.5 mg in 100 mL ethanol.

2.3 Standard Solutions

Standard solutions of the two drugs (0.1 mg mL^{-1}), were prepared by dissolving 10 mg of triclabendazole or ivermectin in methanol to make 100 mL to be used in the HPLC method. A five mg mL^{-1} methanolic solution of triclabendazole and one mg mL^{-1} methanolic solution of ivermectin were used for the densitometric method. One mg mL^{-1} standard solution of ivermectin was prepared in absolute ethanol was utilized by the Vis-spectrophotometric method.

2.4 Procedures

2.4.1 Linearity

2.4.1.1 HPLC method

Aliquots of standard drug solution (0.1 mg mL^{-1}) in methanol equivalent to 0.025-0.500 mg of triclabendazole or 0.01- 0.20 mg of ivermectin were transferred into two sets of 10-mL volumetric flasks and the volume was completed to the mark with methanol. Twenty µL injections from each solution were chromatographed on a Nucleosil column using a mobile phase composed of acetonitrile - methanol - 0.005 M KH_2PO_4 (60:30:10, v/v/v), pH 6, at a flow rate of 1.0 mL min^{-1} and UV detection at 254 nm. The peak area was plotted versus the drug concentration in µg mL^{-1} and the regression parameters were deduced.

2.4.1.2 Densitometric method

Aliquots of standard methanolic solutions of triclabendazole (5 mg mL^{-1}) equivalent to 2.5-25.0 mg or ivermectin (1 mg mL^{-1}) equivalent to 0.5-5.0 mg were transferred into a series of 10-mL volumetric flasks and diluted to the volume with methanol. Ten µL of each solution were applied to TLC plate pre-coated with silica gel 60 F_{254} (10 × 20 cm; Merck, Germany) using a 10 µL microsyringe. The plates were developed in a mobile phase of chloroform: acetone (8: 2, v/v). Then allowed to dry in air and spots were scanned densitometrically at 306 nm for triclabendazole and 245 nm for ivermectin. The calibration curve representing the recorded area under the peak against drug concentration was plotted and the regression equations were computed.

2.4.1.3 Vis-Spectrophotometric method

Aliquots of ivermectin standard solution (1 mg mL^{-1}) equivalent to 0.1-2.0 mg were introduced into a series of 20-mL screw-capped test tubes. One mL of 0.2% alcoholic tetrazolium red solution and 0.3 mL of 0.2 M alcoholic NaOH were added to each tube and volumes were completed to 7 mL with ethanol. The tubes were mixed well and heated in a water bath at 80°C for 20 min, cooled and transferred quantitatively into a series of 10-mL volumetric flasks. Volume was then diluted with ethanol and absorbance of the developed red color was measured 485 nm against a reagent blank. Calibration curve relating the absorbance at 485 nm to the corresponding drug concentration was constructed and the regression equation was calculated.

2.4.2 Assay of laboratory prepared mixtures of the two drugs

2.4.2.1 HPLC method

Different aliquots from standard triclabendazole methanolic solution (0.1 mg mL^{-1}) equivalent to 0.025-0.5 mg drug were mixed with volumes of standard ivermectin solution in methanol (0.1 mg mL^{-1}) equivalent to 0.01-0.2 mg pure drug in 10-mL volumetric flasks. Volumes were completed with methanol, and then 20 µL of each mixture solution was chromatographed on HPLC column following details under "2.4.1Linearity".

2.4.2.2 Densitometric method

Different aliquots from standard triclabendazole methanolic solution (5 mg mL^{-1}) equivalent to 2.5-25.0 mg drug were mixed with volumes of standard ivermectin (1 mg mL^{-1}) equivalent to 0.5-5.0 mg pure drug in 10-mL volumetric flasks. Volumes were completed with methanol and obtained mixtures were then analyzed by densitometric method as described under "2.4.1Linearity".

2.4.2.3 Vis-Spectrophotometric method

Different aliquots from standard triclabendazole ethanolic solution (1 mg mL^{-1}) equivalent to 1.0-2.0 mg drug were mixed with volumes of standard ivermectin solution in ethanol (1 mg mL^{-1}) equivalent to 2.0-1.0 mg pure drug in 20-mL screw-capped test tubes. The ivermectin content in obtained mixtures were analyzed in the presence of triclabendazole as detailed under "2.4.1Linearity".

2.4.3 Application to pharmaceutical formulation

2.4.3.1 HPLC method

The contents of five Trimec Sheep suspensions were thoroughly mixed. A volume of suspension equivalent to 50 mg triclabendazole was pipetted and dissolved in 40 mL methanol by shaking in ultrasonic bath for 10 min, then filtered into 50-mL volumetric flask. Volume was completed with methanol and diluted ten times to obtain a solution labeled to contain 0.1 mg mL^{-1} triclabendazole. Similarly, a volume equivalent to

10 mg ivermectin was transferred and dissolved in 80 mL methanol by shaking in ultrasonic bath for 10 min, then filtered into 100-mL volumetric flask and completed to volume with methanol to obtain a solution labeled to contain 0.1 mg mL^{-1} ivermectin.

Contents of five Triclazole suspensions were well mixed. A volume equivalent to 50 mg triclabendazole was transferred into 50-mL volumetric flask and extracted with methanol as previously mentioned for Trimec Sheep Oral Suspension to obtain a solution labeled to contain 0.1 mg mL^{-1} triclabendazole. Contents of ten Garlec injections were mixed well and volume equivalent to 10 mg ivermectin was transferred into 100-mL volumetric flask, dissolved in 80 mL methanol and completed to volume with methanol to obtain a solution labeled to contain 0.1 mg mL^{-1} ivermectin.

2.4.3.2 Densitometric method

The above-mentioned three pharmaceutical formulations were similarly extracted with methanol as detailed under "2.4.3.1 HPLC method" to obtain a clear solution labeled to contain 5 mg mL^{-1} triclabendazole or 1 mg mL^{-1} ivermectin.

2.4.3.3 Visible-Spectrophotometric method

Trimec sheep suspension and Garlec injection were extracted with dichloromethane and absolute ethanol, respectively; to obtain a clear solution labeled to contain 0.5 mg mL^{-1} ivermectin.

The prepared extracts were analyzed by HPLC, densitometric and Vis-spectrophotometric methods as detailed under "2.4.1Linearity" and the concentration of each drug was calculated from the corresponding regression equation.

3. RESULTS AND DISCUSSION

3.1 HPLC Method

Chromatographic separation of triclabendazole and ivermectin were performed using a Nucleosil column C_{18}. Different systems of mobile phases were tried to separate the two drugs from each other using variable solvents with different ratios as acetonitrile-water, acetonitrile-methanol-water and methanol-water. Best separation was obtained using a mixture of acetonitrile - methanol - 0.005 M KH_2PO_4 (60: 30: 10, v/v/v), pH 6. Different wavelengths (200-400 nm) and flow rates (0.3-1.5 mL min^{-1}) were also tested. Much better detector response was found to be at 254 nm with flow rate of 1.0 mL min^{-1}. The selected conditions yielded satisfactory chromatographic peak resolution in a short analysis time where two sharp resolved peaks at R_t 1.83 ± 0.05 or 4.35 ± 0.19 min. were obtained for triclabendazole and ivermectin, respectively; Fig. 1.

3.1.1 System suitability tests

System suitability [25] was checked where the capacity factor (K´) was 0.68 and 3.00, relative retention time (α) was 4.36, selectivity was 2.38 and resolution (R) was 5.32 and 10.32 and tailing factor (T) was 0.96 and 0.97 for triclabendazole and ivermectin, respectively; indicating that the system was suitable; Table 1.

3.2 Densitometric Method

Initial studies on the two cited drugs were carried out to achieve good simultaneous separation. Different developing systems with different ratios were tried, such as ethyl acetate-methanol-ammonia (33%) (17:2:1, v/v/v), chloroform-methanol (9:1, v/v), toluene-methanol-chloroform-ammonia (33%) (5:3:6:0.1, by volume), methanol-ammonia (9:1.5, v/v) and methanol-water-glacial acetic acid (5:5:0.1, v/v/v); none of them fulfill the purpose. Best separation of triclabendazole and ivermectin was obtained by using a mobile phase of chloroform-acetone (8: 2, v/v). The R_f value of triclabendazole was 0.79 and of ivermectin was 0.33, when visualized under UV lamp at 254 nm. Thus, the chromatogram of the two drugs were measured densitometrically at 306 nm for triclabendazole and 245 nm for ivermectin; Fig. 2.

3.3 Vis-Spectrophotometric Method

A Vis-spectrophotometric method was applied for the determination of ivermectin depending on its reducing character on tetrazolium red to give highly colored red product, namely, formazan with λ_{max} 485 nm. Tetrazolium red was reduced by the hydroxyl group of the lactone ring of ivermectin, where other hydroxyl groups are stabilized either by conjugation or due to steric hindrance; Fig. 3.

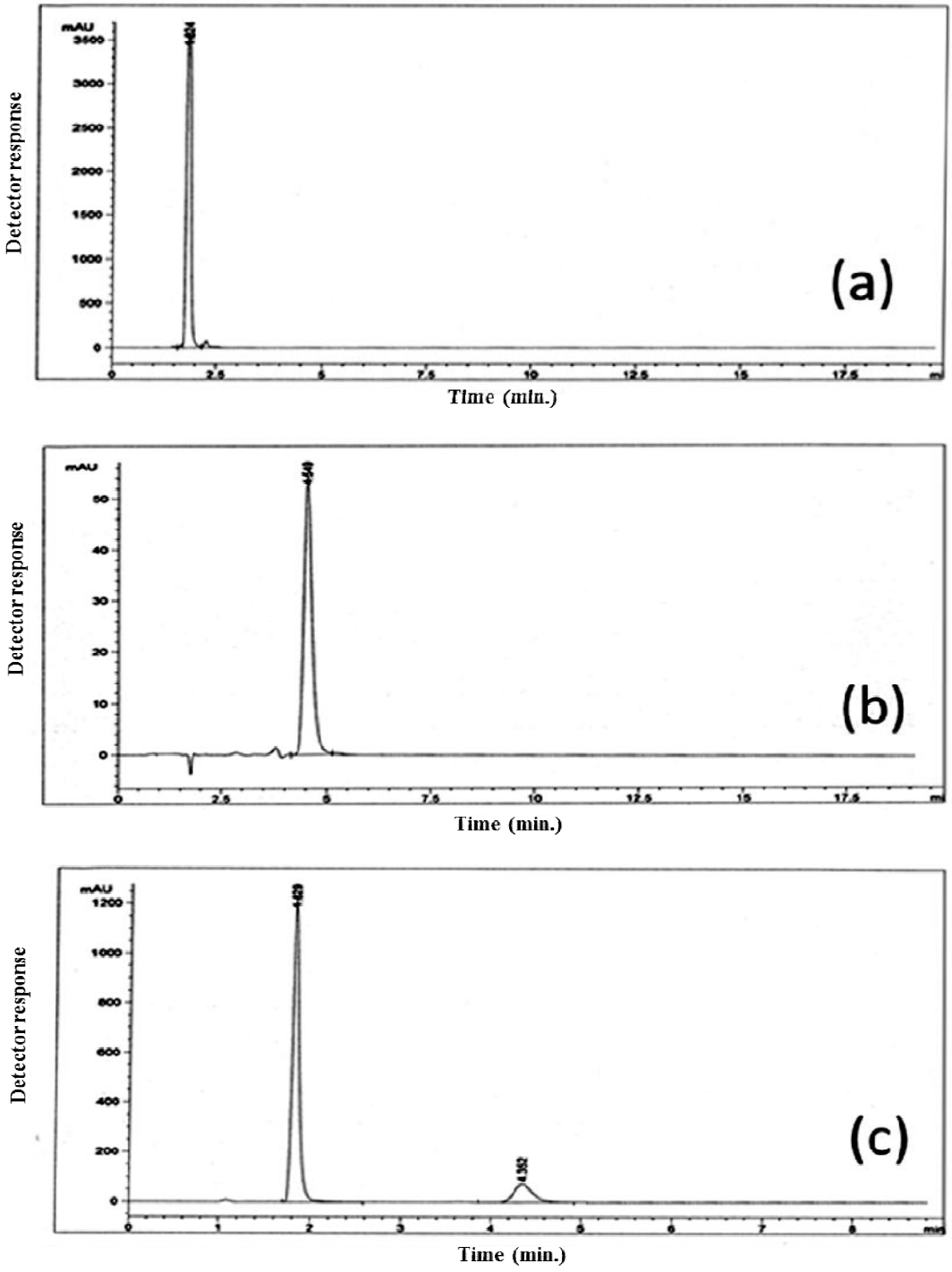
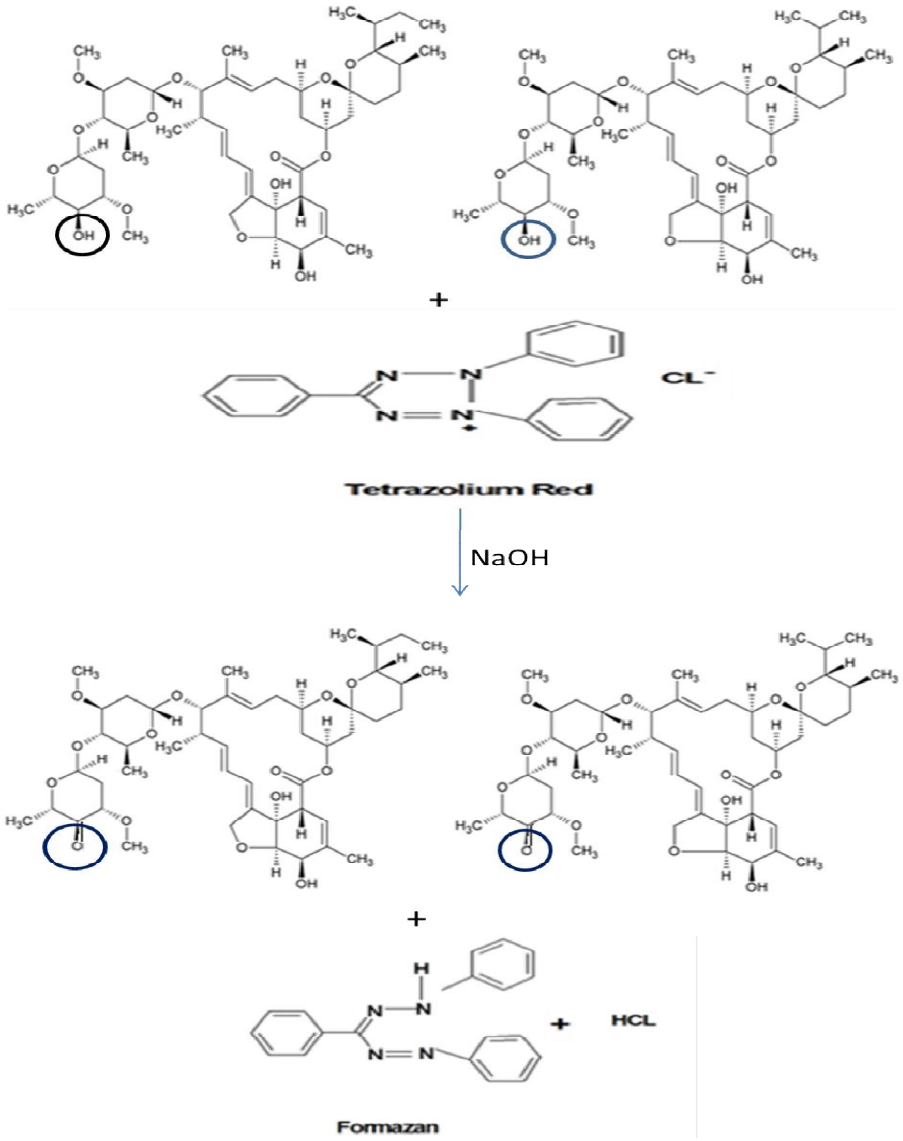


Fig. 1. HPLC chromatogram at 254 nm for:
(a) Triclabendazole (50 µg mL^{-1}).
(b) Ivermectin (20 µg mL^{-1}).
(c) Triclabendazole and Ivermectin mixture in a ratio of 50: 1 µg mL^{-1}

3.3.1 Optimization of the reaction conditions

- Reaction conditions were optimized with regard to the effect of reagents volume, temperature and heating time.
- It was found that alkaline medium was essential for the reaction and 0.1-0.5 of 0.2 M alcoholic NaOH was studied. The most reliable results was obtained with 0.3 mL, thus 0.3 mL was recommended throughout the work.
- Different volumes of 0.2% alcoholic tetrazolium red in the range of 0.1-1.2 were investigated. Best results were obtained upon using 1.0 mL, thus one mL was used for the analysis of the drug.
- Heating was required for the reaction as at room temperature, low and unreliable results were obtained. Different temperatures were tried 40-80°C using thermostatic water bath for about 10-30 min. About 20 min heating at 80°C were recommended during the procedure and the formed color was found to be stable for 50 min.
- Application of molar ratio method [26] showed a ratio of 1:1 (drug: reagent). Thus the suggested reaction mechanism can be illustrated as shown in the following scheme:



Scheme 1. Suggested reaction pathway of reduction of tetrazolium red by ivermectin

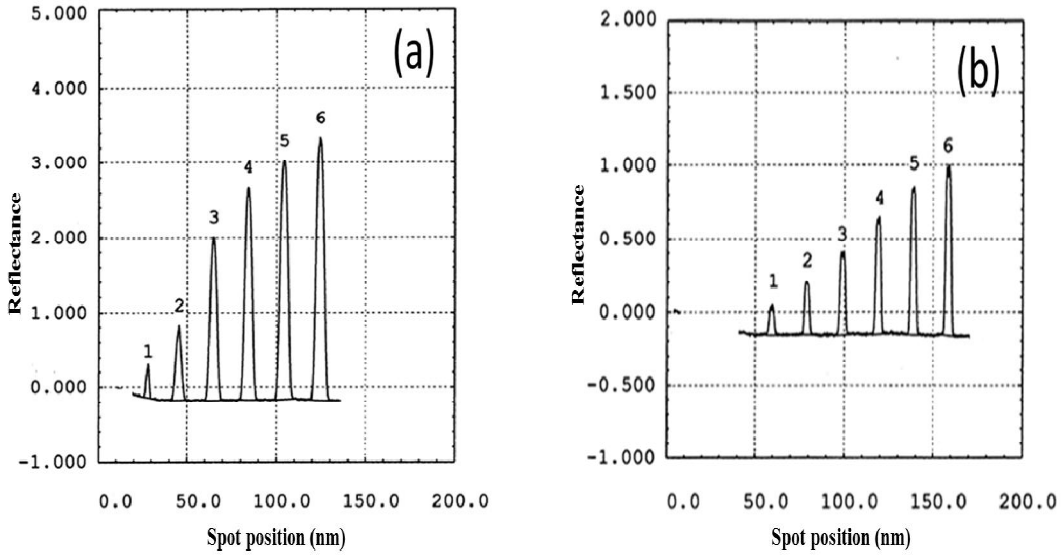


Fig. 2. Densitometric chromatogram of: (a) Triclabendazole (2.5 - 25 µg / spot) at 306 nm. (b) Ivermectin (0.5 - 5 µg / spot) at 245 nm

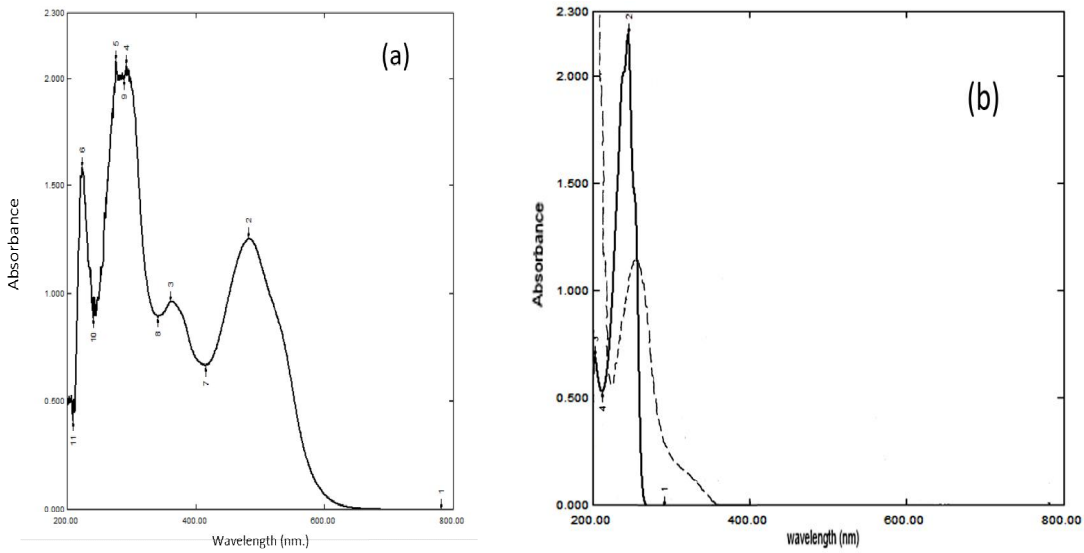


Fig. 3. Absorption spectrum of: (a) Ivermectin–tetrazolium red reaction product (200 µg mL^{-1}). (b) Ivermectin (60 µg mL^{-1}) (—) and tetrazolium red (…) in ethanol

3.4 Method Validation

The three proposed methods were validated according to ICH guidelines [26].

3.4.1 Linearity

A linear correlation was obtained between the peak areas under the curve, peak areas of the separated spots and color intensity at λ_{max} 485 nm and the corresponding drug concentration in the range of 2.5-50.0 µg mL^{-1} and 1-20 µg mL^{-1} by HPLC, 2.5-25.0 µg/spot and 0.5-5.0 µg/spot by densitometric method; for triclabendazole and ivermectin, respectively; or 10-200 µg mL^{-1} for ivermectin by Vis-spectrophotometric method; Figs. (4-8). The regression parameters were calculated and presented in Table 2.

3.4.2 Accuracy and precision

The accuracy of the proposed methods was tested using three concentrations of the cited drugs within the linearity range and found to be 100.11%, 100.38% for triclabendazole and 100.05%, 100.61% for ivermectin. Precision was

also evaluated by calculating the intraday RSD%, which ranged between 0.67 and 1.85% and interday RSD%, 0.66 - 1.77% over a period of two months for triclabendazole. For ivermectin, intraday RSD%, was 0.91 - 1.68% while interday RSD%, 0.29 - 1.77%. This indicated the repeatability and reproducibility of the proposed methods; Table 2.

3.4.3 Selectivity

It was revealed by analyzing laboratory prepared mixtures of triclabendazole together with ivermectin in different ratios (1:2, 1:1, 2:1 and 50:1, respectively) by the HPLC and densitometric methods for simultaneous determination of the two mentioned drugs without any interference; as indicated by recoveries of 100.53% - 99.94% ± 0.97 - 1.60 and 100.03% - 98.77% ± 0.82 - 0.72 for the two drugs, respectively; Table 2. While the Vis-spectrophotometric method was valid for the determination of ivermectin the presence of triclabendazole in a ratio of (1:2, 1:1, and 2:1, respectively) with mean recovery of 100.86% ± 0.93; Table 3.

The selectivity of the proposed methods was further evaluated by successful analysis of the cited drugs in their pharmaceutical formulations. The mean recoveries were 100.85% ± 0.95 and 100.08% ± 1.33 for triclabendazole in Trimec and Triclazole suspensions by HPLC and 100.68% ± 0.77 and 101.13% ± 1.73 by densitometry; respectively. For ivermectin The mean recoveries were 98.65% ± 1.61 and 101.46% ± 1.56 in Trimec suspension and Garlec injection by HPLC, 101.03% ± 1.49 and 99.79% ± 1.23 by densitometry and 101.94% ± 1.22 and 101.74% ± 1.57 by Vis-spectrophotometric, respectively; Table 3. It is noteworthy to mention that the HPLC and densitometric methods was also successfully applied for the determination of the cited drugs in their pharmaceutical formulations by preparing two solutions from their pharmaceutical formulations previously mentioned, one for the analysis of triclabendazole and the other for ivermectin analysis because both drugs cannot be analyzed in a single prepared solution containing a ratio of 50:1 of triclabendazole and ivermectin as in the Trimec suspension. Moreover, dichloromethane was used to extract ivermectin from Ttrimec suspension for its analysis by Vis-spectrophotometry to remove interference of high concentration of triclabendazole which is insoluble in dichloromethane.

Validity of the proposed methods was further assessed by applying the standard addition technique; the mean recoveries were 100.87% ± 1.11 and 100.80% ± 0.90 for triclabendazole in Trimec and Triclazole suspensions by HPLC and 100.89% ± 0.54 and 98.98% ± 0.50 by densitometry; respectively. For ivermectin The mean recoveries were 100.61% ± 0.82 and 99.65% ± 1.32 in Trimec suspension and Garlec injection by HPLC, 99.57% ± 0.91 and 100.12% ± 0.70 by densitometry and 100.80% ± 0.92 and 100.68% ± 1.66 by Vis-spectrophotometry, respectively; Table 4. These results were statistically compared with those obtained from the manufacturer method which is a direct UV spectrophotometric method for triclabendazole, and HPLC method for ivermectin [27]. As shown in Table 4, calculated *t*- and *F*-values were less than theoretical ones, indicating that there was no significant difference between the proposed and reported methods.

3.4.4 Robustness

No significant change in the system suitability parameters was obtained upon introduction of small variations in acetonitrile volume (58-61 mL) or methanol volume (29-32 mL), where the resolution between triclabendazole and ivermectin was not altered and gave RSD% did not exceed 1.85% for triclabendazole and 1.52% for ivermectin; illustrating the robustness of the HPLC method. For the densitometric method, upon introduction of small variations in chloroform volume (7.9-8.1 mL) or acetone volume (1.9-2.1 mL), the R_f value gave RSD% not exceed 1.61% and 1.72% for triclabendazole and ivermectin, respectively. Robustness of the Vis-spectrophotometry was assessed by studying the influence of small variations in the volume of NaOH (0.3-0.5 mL). It was observed that no significant change in the obtained results, the RSD% was less than 0.34%; the method suits best.

3.4.5 Stability of standard solution

The stability of triclabendazole methanolic solutions (0.1 mg mL^{-1} and 5 mg mL^{-1}) and ivermectin solutions (0.1 mg mL^{-1} and 1 mg mL^{-1} in methanol and 1 mg mL^{-1} in ethanol) were evaluated by HPLC, densitometric, Vis-spectrophotometric methods; respectively. This was carried out through storing on laboratory bench and in the refrigerator at 4°C. The solutions were found to be stable for two weeks either at room temperature or in refrigerator.

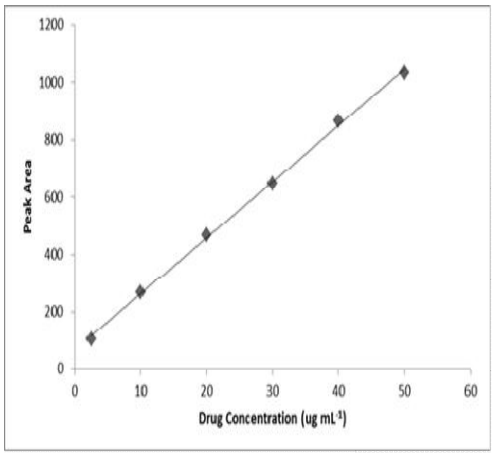


Fig. 4. Calibration curve of peak area at 254 nm to the corresponding concentration of triclabendazole

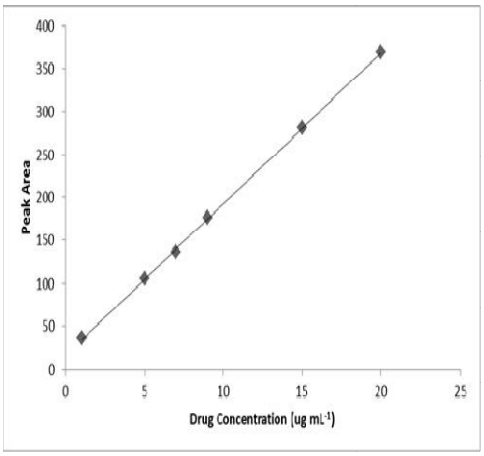


Fig. 5. Calibration curve of peak area at 254 nm to the corresponding concentration of ivermectin

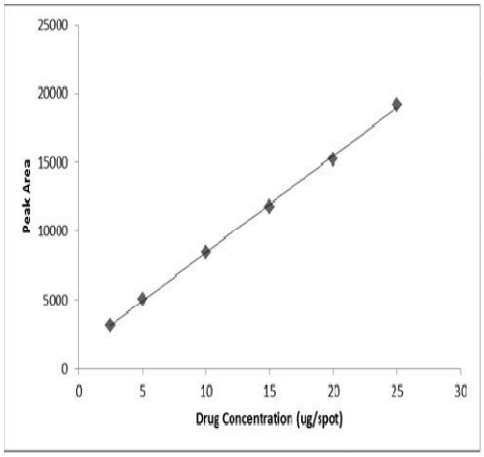


Fig. 6. Calibration curve of peak area at 306 nm to the corresponding concentration of triclabendazole

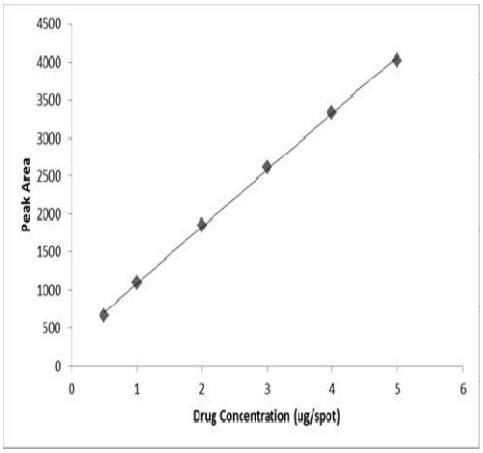


Fig. 7. Calibration curve of peak area at 245 nm to the corresponding concentration of ivermectin

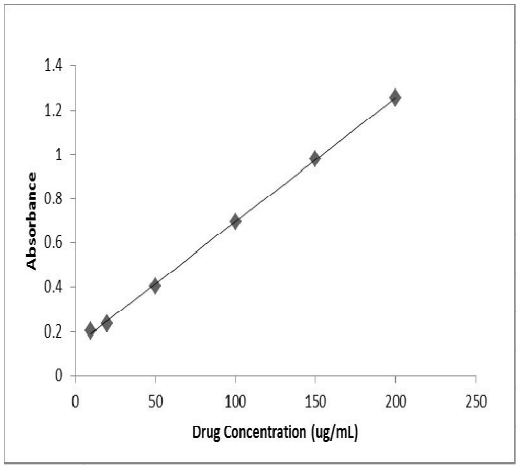


Fig. 8. Calibration curve of absorbance of ivermectin reaction product with tetrazolium red to drug concentration at 485 nm

Table 1. Parameters of system suitability test of the proposed HPLC method for triclabendazole and ivermectin mixtures

Parameter	Obtained value		
	Triclabendazole R_t=1.83	**Ivermectin R_t=4.35**	**Reference value [148]**
Column capacity (K´)	0.69	3.01	0.5 -10 acceptable
Relative retention time (α)	4.36		>1
Resolution (R)	5.32	10.32	R >1.5
Tailing factor (T)	0.96	0.97	T=1 for a typical symmetric peak
Column efficiency (N)	3207	2354	Increase with the efficiency of the separation
HETP	0.039	0.053	The smaller the value, the higher the column efficiency

The bandwidth is 0.08 min for triclabendazole and 0.21 min for ivermectin.

Table 2. Regression parameters and assay validation results for the determination of triclabendazole and ivermectin by the proposed methods

Parameters	Triclabendazole		Ivermectin		
	HPLC method	**Densitometric method**	**HPLC method**	**Densitometric method**	**Vis-Spectrophotometric method**
linearity range	2.5 – 50.0 µg mL^{-1}	2.5 – 25.0 µg/spot	1.0 – 20.0 µg mL^{-1}	0.5 – 5.0 µg/spot	10.0 – 200.0 µg mL^{-1}
Regression parameters					
Slope ± S.D	19.56 ± 0.300	701.57 ± 9.926	17.537 ± 0.162	743.11 ± 9.372	0.0056 ± 6.599
Intercept ± S.D.	66.144 ± 9.093	1428.9 ± 150.606	17.483 ± 1.848	345.21 ± 28.442	0.1328 ± 0.007
S.D. of residual	12.118	193.550	2.507	36.552	0.011
Correlation coefficient	0.9991	0.9992	0.9997	0.9994	0.9994
Accuracy (R%)	100.11	100.38	100.08	100.05	100.61
Precision (RSD%, n=9)					
Intraday	0.67 – 1.68	0.90 – 1.85	1.03 – 1.29	1.23 – 1.57	0.91 – 1.68
Interday	1.34 – 1.56	0.66 – 1.77	1.40 – 1.53	0.29 – 0.53	1.08 – 1.77

Table 3. Determination of triclabendazole and ivermectin in mixtures using the proposed methods

Drugs ratio	HPLC method				Densitometric method				Vis-Spectrophotometric method		
	Triclabendazole (µg mL $^{-1}$)	Ivermectin (µg mL $^{-1}$)	Recovery % of Triclabendazole	Recovery % of Ivermectin	Triclabendazole (µg/spot)	Ivermectin (µg/spot)	Recovery % of Triclabendazole	Recovery % of Ivermectin	Triclabendazole (µg mL $^{-1}$)	Ivermectin (µg mL $^{-1}$)	Recovery % of Ivermectin
50:1	50	1	100.85	100.37	25	0.5	100.99	99.68	-	-	-
1:1	20	20	101.31	99.22	2.5	2.5	98.72	98.95	100	100	101.11
2:1	20	10	99.11	99.51	5	2.5	101.61	98.00	200	100	101.64
1:2	10	20	100.87	101.02	2.5	5	98.43	98.45	100	200	99.839
	Mean% ± SD		100.53 ± 0.97	100.03 ± 0.82	**Mean% ±SD**		99.94 ± 1.60	98.77 ± 0.72	**Mean% ± SD**		100.86 ± 0.93

Table 4. Statistical analysis of determination of triclabendazole and ivermectin in pharmaceutical formulation by the proposed methods in comparison with the manufacture method [27]

Parameters	Triclabendazole						Ivermectin							
	HPLC method		Densitometric method		Manufacturer method [27]		HPLC method		Densitometric method		Vis-Spectrophotometric method		Manufacturer method [27]	
	Trimec	Triclazole	Trimec	Triclazole	Trimec	Triclazole	Trimec	Garlec	Trimec	Garlec	Trimec	Garlec	Trimec	Garlec
Mean %	100.85	100.08	100.68	101.13	99.34	101.06	98.65	101.46	101.03	99.79	101.936	101.739	100.35	100.34
S.D.	0.953	1.334	0.770	1.728	1.141	0.89	1.609	1.561	1.494	1.227	1.222	1.573	1.525	0.716
Variance	0.909	1.779	0.593	2.984	1.301	0.787	2.591	2.436	2.235	1.505	1.493	2.473	2.326	0.513
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5
t – test	2.281	1.367	2.176	0.083	-	-	1.714	1.454	0.718	0.864	1.817	1.809	-	-
F – test	1.431	2.260	2.194	3.792	-	-	1.114	4.749	1.041	2.934	1.558	4.821	-	-
Standard addition Mean%± S.D.	100.87 ± 1.11	100.80 ± 0.90	100.89 ± 0.54	98.98 ± 0.50	-	-	100.61 ± 0.82	99.65 ± 1.32	99.57 ± 0.91	100.12± 0.70	100.80 ± 0.92	100.68 ± 1.66	-	-

The theoretical t- and F- values at P=0.05 were 2.31 and 6.39; respectively.

Ref. [27] UV- measurements of triclabendazole at 306 nm in methanol and HPLC method for ivermectin using C_{18} column and UV detection at 245 nm

4. CONCLUSION

Two methods; HPLC and densitometry have been developed for the simultaneous determination of triclabendazole and ivermectin in veterinary preparation. The HPLC method was rapid, where the total analytical run time for both drugs was less than 6 min. Both methods were bonuses for routine analysis of triclabendazole and ivermectin singly, in their mixtures or in their pharmaceutical formulations due to saving time, high sensitivity and selectivity. The third method was a Vis-spectrophotometric one which was used for the analysis of ivermectin only. Validation of the proposed methods was carried out according to ICH guidelines; the methods proved to be accurate, precise, robust and rugged.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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