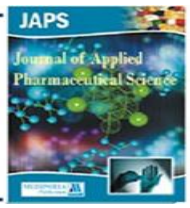
Journal of Applied Pharmaceutical Science
Available online at www.japsonline.com



ISSN: 2231-3354
Received on: 04-10-2011
Revised on: 02:11:2011
Accepted on: 23-11-2011

Spectrophotometric determination of triclabendazole by acid-dye complexation method in bulk and pharmaceutical formulation

Nesrin K. Ramadan, Afaf O. Mohamed, Sara E. Shawky and Maissa Y. Salem

Nesrin K. Ramadan, Maissa Y. Salem
Analytical Chemistry Department, Faculty of pharmacy, Cairo University, Kasr El-Aini St,11562 Cairo-Egypt.

Afaf O. Mohamed, Sara E. Shawky
National Organization for Drug Control and Research (NODCAR), Giza-Egypt.

For Correspondence
Nesrin K. Ramadan
Analytical Chemistry Department, Faculty of pharmacy, Cairo University, Kasr El-Aini St,11562 Cairo-Egypt.

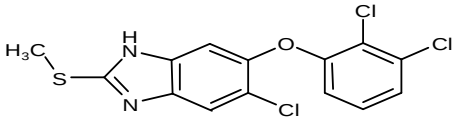
ABSTRACT

Two simple, accurate and sensitive spectrophotometric methods were developed for the determination of triclabendazole in bulk and pharmaceutical formulation. These methods were based on the formation of ion-pair association complex (1:1) with bromocresol purple (BCP) and bromophenol blue (BPB). The coloured products were extracted into chloroform and measured spectrophotometrically at 420 nm. Linearity ranges were 20 – 100 µg/ml for BCP and 10 – 100 µg/ml for BPB, the mean percentage recoveries were 99.61±1.152% and 99.60±0.986% for BCP and BPB respectively. Statistical comparison between the results obtained by these methods and the manufacturer's method was done, and no significance difference was obtained.

Keywords: Triclabendazole, spectrophotometry, bromocresol purple, bromophenol blue.

INTRODUCTION

Triclabendazole, 5-Chloro-6-(2,3-dichlorophenoxy)- 2-(methylthio)benzimidazole (Sweetman, 2009).



Triclabendazole,
Molecular formula = $C_{14}H_9Cl_3N_2OS$; Molecular weight = 359.7

Triclabendazole is a benzimidazole anthelmintic drug used in veterinary medicine for the treatment of fascioliasis. It is also increasingly being used in the treatment of human fascioliasis, and is under investigation for the treatment of human paragonimiasis (Sweetman, 2009). Literature survey reveals few analytical methods for quantification of the drug in pure form, in pharmaceutical dosage forms, in biological fluids and in the presence of its metabolites including continuous wavelet transform and derivative spectrophotometry (Dinc *et al.*, 2009) and HPLC methods (Zhou *et al.*, 2005) (Mottier *et al.*, 2004) (De-Ruyck *et al.*, 2002) (Takeba *et al.*, 2002) (Negro *et al.*, 1992) (Marti *et al.*, 1990) (Bull and Shume, 1987) (Lehr and Damm, 1986) (Alvinerie and Galtier, 1986).

This paper presents simple and accurate methods for the determination of triclabendazole by acid-dye complexation methods in its pure powder and in suspension forms.

EXPERIMENTAL

Instruments

Shimadzu UV-2400 PC Series Spectrophotometer with two matched 1cm quartz cell.

Reagents

Chloroform, Bromo-phenol blue (1×10^{-3} M) [BDH, UK]. Hydrochloric acid (0.1M aqueous solution), Acetic acid (0.1M aqueous solution) [ADWIC. Cairo, Egypt]. Sulphuric acid (0.1M aqueous solution), Nitric acid (0.1M aqueous solution) [Sigma-Aldrich, Germany]. Bromo-cresol purple (1×10^{-3} M) [WINLAB, UK].

Samples

Reference sample

Triclabendazole pure sample was kindly supplied by Pharma Swede, Egypt. Its purity was found to be 99.20±0.750% according to the manufacturer's method [Medco- Erp limited, Holland. Registrations files from National Organization of Drug Control and Research, Registration Files Section.].

Pharmaceutical formulation: Triclazole 10% oral suspension B.N. 805646 was supplied by EVA Pharma for Pharmaceuticals & Medical Appliance, Egypt. Each 1 ml is claimed to contain 100 mg of triclabendazole.

Standard solutions

Triclabendazole stock solution: (2.5 mg/ml) in chloroform. Triclabendazole stock solution for stoichiometry of the reaction (1×10^{-3} M) in chloroform: prepared by dissolving 35.9 mg in a 100-ml volumetric flask, the volume was completed to the mark with chloroform.

Procedures

Linearity

Using BCP

Aliquots (0.2, 0.3, 0.4, ……., 1 ml) of the drug working standard solution (2.5 mg/ml) were transferred into a series of 50-ml separating funnels, 6 ml of BCP solution (1×10^{-3} M) and 2 ml of 0.1M HCl were added, the complex was extracted two times with 10 ml chloroform. The solution was shaken for 1 min each time and the chloroform layer was passed through anhydrous sodium sulphate into a 25-ml volumetric flask, the volume was completed to the mark with chloroform. The absorbance was measured at 420 nm against blank constructed the same as the experiment omitting the addition of the drug. Linear calibration curve was constructed relating the absorbance to the corresponding concentration of triclabendazole and the corresponding regression equation was computed.

Using BPB

Aliquots (0.1, 0.2, 0.3, ……., 1 ml) of the drug working standard solution (2.5 mg/ml) were transferred into a series of 50-ml separating funnels, 6 ml of BPB solution (1×10^{-3} M) and 1 ml of 0.1M HCl were added, the complex was extracted two times with 10 ml chloroform. The solution was shaken for 1 min each time and the chloroform layer was passed through anhydrous sodium sulphate into a 25-ml volumetric flask, the volume was completed to the mark with chloroform. The absorbance was measured at 420 nm against blank constructed the same as the experiment omitting the addition of the drug. Linear calibration curve was constructed relating the absorbance to the corresponding concentration of triclabendazole and the corresponding regression equation was computed.

Accuracy

The accuracy of the results was checked by applying the previously mentioned procedures under linearity for different concentration of pure triclabendazole within the linearity ranges. The concentrations of the drug were calculated from the corresponding regression equation. The mean recovery percentages and relative standard deviations were then calculated.

Precision

Repeatability

Three concentrations of triclabendazole stock standard solution (20, 40 and 60 µg/ml) were analyzed three times each, intra-day, using the previously mentioned procedures under subsection of ***Linearity.*** The mean recovery percentages and relative standard deviations were then calculated.

Intermediate precision

The above mentioned triclabendazole samples were analyzed on three successive days using the procedures stated under subsection of ***Linearity.*** The mean recovery percentages and relative standard deviations were then calculated.

Application of the proposed method for the determination of triclbendazole in its pharmaceutical formulation

The contents of the Triclazole 10% oral-suspension bottle were shacked well then 2.5 ml of the suspension equivalent to 250 mg triclabendazole was quantitatively transferred into a 100-ml beaker, 50 ml chloroform was added. The beaker was covered with glass watch, the solution was stirred for 30 minutes using a magnetic stirrer, and then filtered into a 100-ml volumetric flask, the residue was washed three times each with 10 ml chloroform and filtered, the collected filtrates were quantitatively transferred to the volumetric flask, and the volume was completed to the mark with chloroform to prepare solution of concentration of (2.5 mg/ml). 0.5 ml was transferred into a 50-ml separating funnel. Then the procedure was completed as detailed under linearity. The concentration of triclabendazole was calculated using the corresponding regression equation.

Optimization of the reactions conditions

The optimization of the methods was carefully studied to achieve complete reaction formation, highest sensitivity and maximum absorbance. Reaction conditions of the ion-pair complex were found by studying with preliminary experiments such as type of acid, volumes of the dye, volumes of the acid and the stability of ion-pair complexes.

Effect of different acids

Different acids were tested such as 0.1M hydrochloric acid, 0.1M sulphuric acid, 0.1M nitric acid and 0.1M Acetic acid. And the absorbance reading of triclabendazole ion-pair complexes were examined.

Effect of acid concentration

Different volumes of 0.1M hydrochloric acid ranging from (0.5-4 ml) for BCP and from (0.5-5 ml) for BPB methods were tested. And the absorbance of triclabendazole ion-pair complexes was recorded.

Effect of dye concentration

The effect of dye concentration on the intensity of the color developed at the selected wavelengths was ascertained using different volumes of the reagents ranging from (1-8 ml) for both BCP and BPB methods and the absorbance of triclabendazole ion-pair complexes were recorded.

Stability of the complexes

The stability of the developing colors was studied by measuring the absorbance at different time intervals (0-60 min).

Stoichiometric relationship

Job's method of continuous variation (Yoe and Jones 1944) of equimolar solutions was employed: a 1 x 10^{-3} M standard solution of drug and 1 x 10^{-3} M solution of BCP and BPB, respectively, were used. A series of solutions was prepared in which the total volume of drug and reagent was kept at 10 ml for BCP and BPB, respectively. The absorbance was measured at the optimum wavelength. The molar ratio of the reagents (drug: dye) in the ion-pair complexes was determined by the continuous variations method (Job's method)

RESULTS AND DISCUSSION

Extractive spectrophotometric methods are popular for their sensitivity in the assay of drugs and, therefore, ion-pair extractive spectrophotometry has received considerable attention for the quantitative determination of many pharmaceutical compounds (Harikrishna *et al.*, 2008) (Ashour *et al.*, 2006) (Basavaiah and Charan, 2004). However, no reports have appeared dealing with the extractive spectrophotometric method for the determination of triclabendazole in pure powdered form or in pharmaceutical formulations. Therefore, this present work proposes two simple and sensitive extractive spectrophotometric methods for the assay of triclabendazole. The methods were based on that the drug in its protonated form reacts with BCP and BPB to form yellow chloroform-extractable complex. The absorption spectra of the extracted complex were recorded over the range 300-600 nm. The complex showed a maximum absorbance at 420 nm, which could be used as the wavelength for determination, **(Figs-1, 2)**.

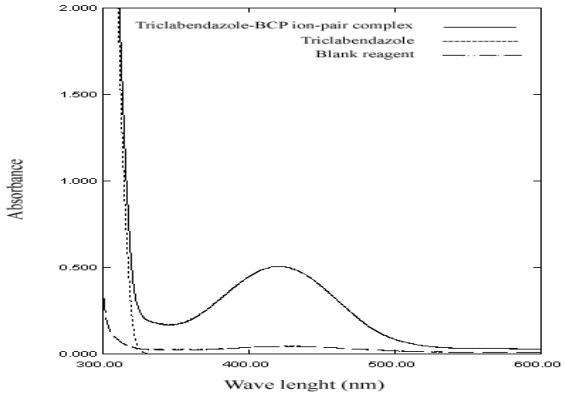


Fig 1: Absorption spectra of: triclabendazole (50µg/ml) in chloroform, triclabendazole-BCP ion-pair complex (50µg/ml) in chloroform and blank reagent.

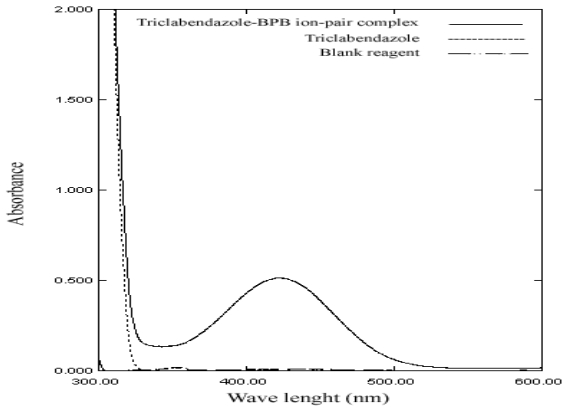
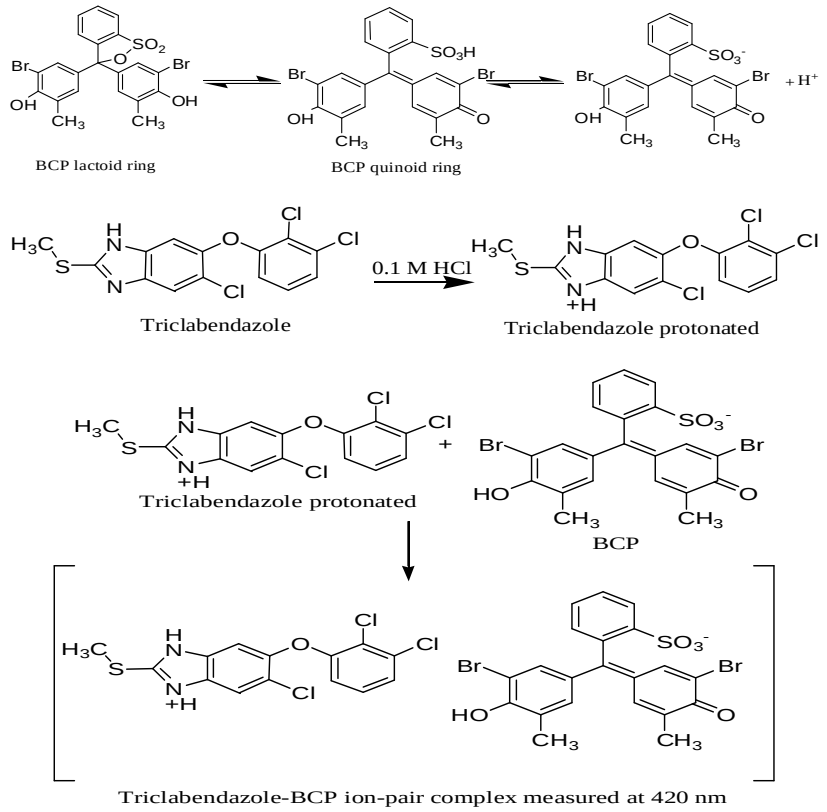
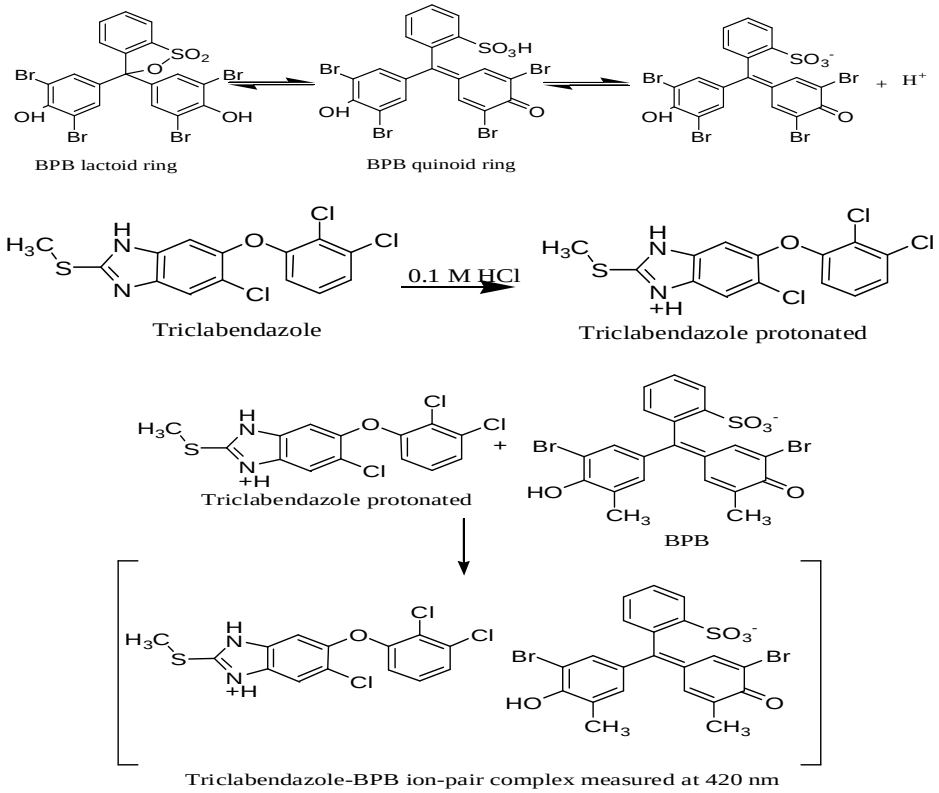


Fig 2: Absorption spectra of: triclabendazole (50µg/ml) in chloroform, triclabendazole-BPB ion-pair complex (50µg/ml) in chloroform and blank reagent.

The possible reaction mechanisms for the formation of ion-pair complex by BCP and BPB are shown in **Schemes-1, 2** respectively. The reaction conditions were optimized, different acids were tried and the results showed that the highest absorbance for both methods was achieved by using 0.1M hydrochloric acid, **(Fig-3)**. Maximum color intensity was obtained upon using 2 ml of 0.1M hydrochloric acid by using BCP as ion pair dye and 1 ml of 0.1M hydrochloric acid by using BPB as ion pair dye, **(Fig-4)**. 6 ml of 1×10^{-3} M of both reagents were optimum, **(Fig-5)**. The absorbance of the ion pair complexes remains stable for at least 60 min, **(Fig 6)** The stoichiometry of reaction of triclabendazole and the two dyes was suggested as shown in **schemes-1, 2** and it was proved by applying job's method using equimolar concentrations of the drug and the dyes .It was found that the reaction proceeds in a molar ratio of 1:1 in both methods, **(Figs-7, 8)**.



Scheme (1): The possible reaction mechanism of formation of triclabendazole-BCP ion-pair complex.



Scheme (2): The possible reaction mechanism of formation of triclabendazole-BPB ion-pair complex.

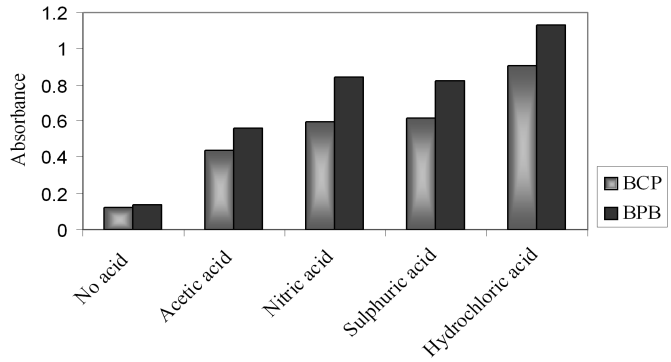


Fig 3: Effect of acid type on the absorbance of (100 µg/ml) of triclabendazole ion-pair complex in chloroform at 420 nm.

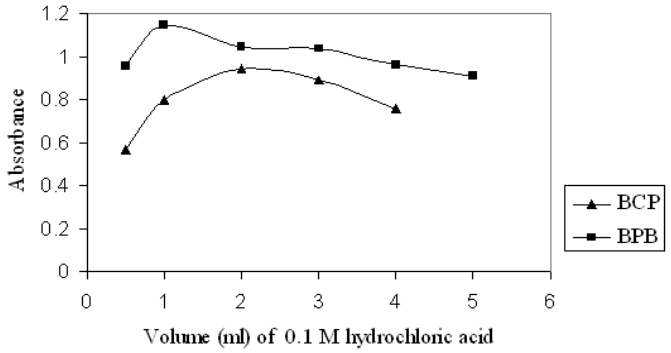


Fig 4: Effect of volume of 0.1M HCl on the absorbance of (100 µg/ml) of triclabendazole ion-pair complex in chloroform at 420 nm.

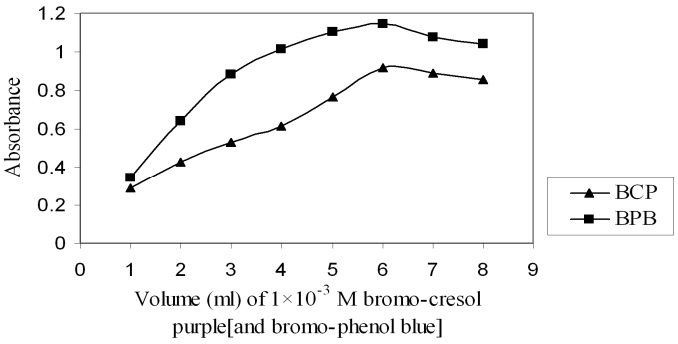


Fig 5: Effect of dye concentration on the absorbance of (100 µg/ml) of triclabendazole ion-pair complex in chloroform at 420 nm.

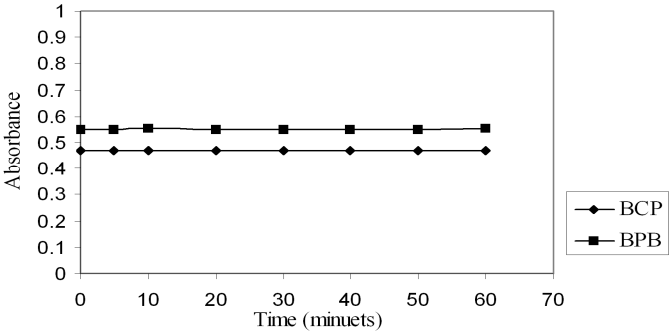


Fig 6: Effect of time on the stability of (50 µg/ml) of triclabendazole ion-pair complex in chloroform at 420 nm.

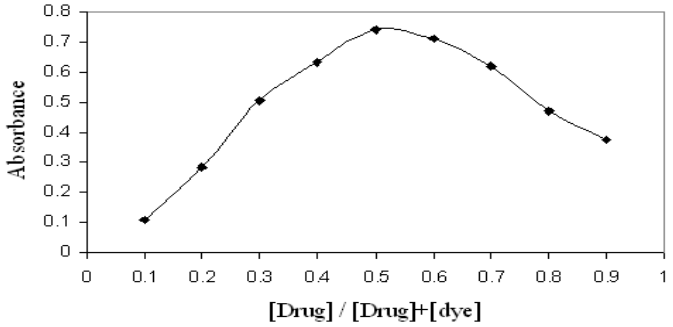


Fig 7: Job's plot for stoichiometric ratio between triclabendazole and BCP (1×10^{-3} M).

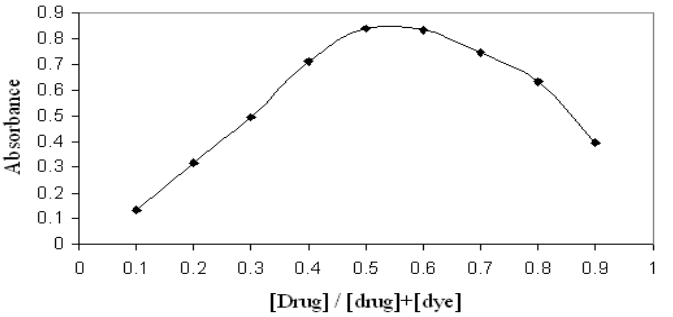


Fig 8: Job's plot for stoichiometric ratio between triclabendazole and BPB (1×10^{-3} M).

Linear relationship was obtained between the absorbance and the corresponding concentration of triclabendazole in the range of (20-100 µg/ml) by using BCP and (10-100 µg/ml) by using BPB. The regression equations were computed and found to be:

$A_1 = 0.0093\ C - 0.0046$ r = 0.9992 using BCP
$A_2 = 0.0121\ C - 0.0408$ r = 0.9991 using BPB

Where A_1 is the absorbance of triclabendazole-BCP ion-pair complex, A_2 is the absorbance of triclabendazole-BPB ion-pair complex, C is the concentration µg/ml and r is the correlation coefficient.

The proposed method was successfully applied for the determination of the drug in pure powdered form with mean percentage recoveries of 99.61±1.152% for BCP and 99.60±0.986% for BPB, **(Table-1)**. The proposed method was successfully applied for the determination of triclabendazole in its pharmaceutical dosage form. And its validity was assessed by applying the standard addition technique, **(Table-2)**. The results obtained by applying the proposed method was statistically compared with those obtained by the manufacturer's method, the values of the calculated t and F were less than the tabulated ones which revealed that there is no significant difference between them, **(Table-3)**. Validation of the proposed acid-dye methods for the determination of triclabendazole was made by measuring concentration range, accuracy, precision, repeatability, intermediate precision and linearity. Results obtained are depicted in **Table-1**.

Table-1. Results of validation parameters of the responses and the regression equations obtained by the proposed methods.

Parameters	BCP	BPB
Slope [a]	0.0093	0.0121
Intercept [a]	-0.0046	-0.0408
Correlation coefficient	0.9992	0.9991
Concentration range (µg/ml)	20 – 100	10 – 100
Average accuracy (%)	99.61	99.60
S.D.	1.147	0.982
R.S.D. %	1.152	0.986
Repeatability [b] %± R.S.D.	99.90±0.450	100.17±0.415
Intermediate precision [c] %± R.S.D.	99.75±0.383	100.20±0.229

[a] Results of five determinations
[b] n = 3×3
[c] n = 3×3

Table-3. Statistical analysis between the results obtained for the determination of triclabendazole in pure samples by the proposed methods and those obtained by the manufacturer's method.

Item	BCP	BCP	Manufacturer's method*
Mean	99.61	99.60	99.20
S.D.	1.147	0.982	0.744
R.S.D%	1.152	0.986	0.750
Variance	1.316	0.964	0.554
n	9	10	5
Student's t	0.713(2.179)	0.797(2.160)	
F test	2.375(4.82)	1.740(4.77)	

Figures in parentheses are the corresponding tabulated values at p = 0.05
* Manufacturer's method was a HPLC, mobile phase water: acetonitrile (10:90 by volume) respectively, UV set at 258 nm and C18 column.

Table-2 Quantitative determination of triclabendazole in pharmaceutical formulation by the proposed methods and results of application of standard addition technique.

Triclazole oral suspension 10% B.N 805646	BCP			
	Found % [a]	**Claimed amount taken (µg/ml)**	**Standard added (µg/ml)**	**Recovery %** [b] **of added**
	98.56	50	20	99.41
	98.97	50	30	98.11
	98.77	50	40	101.06
	99.18	50	50	99.58
	98.56			
	99.18			
Mean	98.87			99.54
S.D.	0.283			1.209
R.S.D. %	0.286			1.215
	BPB			
Triclazole oral suspension 10% B.N 805646	**Found %** [a]	**Claimed amount taken (µg/band)**	**Standard added (µg/band)**	**Recovery %** [b] **of added**
	98.74	50	20	100.50
	100.18	50	30	100.63
	100.54	50	40	99.28
	101.08	50	50	100.91
	100.54			
	99.28			
Mean	100.06			100.33
S.D.	0.877			0.722
R.S.D. %	0.877			0.719

[a] Average of six determinations
[b] Average of six determinations

REFERENCES

Alvinerie M., Galtier P. Assay of triclabendazole and its main metabolites in plasma by high-performance liquid chromatography. J Chromatogr Biomed Appl. 1986; 47(2 (J. Chromatogr., 374)): 409-414.

Ashour S., Chehna MF., Bayram R. Int. Spectrophotometric determination of alfuzosin HCl in pharmaceutical formulations with some sulphonephthalein dyes J Biomed Sci. 2006; 2: 273-278.

Basavaiah K., Charan VS. Ion-pair complexometric determination of cyproheptadine hydrochloride using bromophenol blue. Sci Asia. 2004; 30**:** 163-170.

Bull MS., Shume GRE. Rapid high-performance liquid-chromatographic procedure for the determination of triclabendazole and its metabolites in sheep plasma. J Pharm Biomed Anal. 1987; 5(5): 527-531.

De-Ruyck H., Daeseleire E., De-Ridder H., Van-Renterghem R. Development and validation of a liquid chromatographic-tandem electrospray mass spectrometric multiresidue method for anthelmintics in milk. J Chromatogr A. 2002; 976(1-2): 181-194.

Dinc E**.,** Pektas G., Baleanu D. Continuous Wavelet Transform and Derivative Spectrophotometry for the Quantitative Spectral Resolution of a Mixture containing Levamizole and Triclabendazole in Veterinary Tablets. Reviews in Analytical Chemistry. 2009; 28(2): 79-92.

Harikrishna K., Nagaralli BS., Seetharamappa. Extractive spectrophotometric determination of sildenafil citrate (viagra) in pure and pharmaceutical formulations J Food Drug Anal. 2008; 16: 11-17.

Lehr KH., Damm P. Simultaneous determination of fenbendazole and its two metabolites and two triclabendazole metabolites in plasma by high-performance liquid chromatography. J Chromatogr Biomed Appl. 1986; 55(J. Chromatogr., 382): 355-360.

Marti AM., Mooser AE., Koch H. Determination of benzimidazole anthelmintics in meat samples. J Chromatogr. 1990; 498(1): 145-157.

Mottier L., Moreno L., Alvarez L., Virkel G., Lanusse C. Measurement of triclabendazole and its metabolites in liver flukes: method development and full validation. J Pharm Biomed Anal. 2004; 35(5): 991-999.

Negro A., Alvarez-Bujidos ML., Ortiz AI., Cubria JC., Mendez R., Ordonez D. Reversed-phase ion-pair high-performance liquid-chromatographic determination of triclabendazole metabolites in serum and urine. J Chromatogr Biomed Appl. 1992; 114(1 (J. Chromatogr., 576)): 135-141.

Sweetman SC. Martindale "The Complete Drug Reference", 36th ed. The Pharmaceutical Press, London, UK, 2009; 157.

Takeba K., Fujinuma K., Sakamoto M., Miyazaki T., Oka H., Itoh Y., Nakazawa H. Simultaneous determination of triclabendazole and its sulfoxide and sulfone metabolites in bovine milk by high-performance liquid chromatography. J Chromatogr A. 2002; 882(1-2): 99-107.

Yoe JH., Jones AL. Ind Eng Chem Anal. 6th ed. 1944; 111.

Zhou H., Tai YP., Sun CR., Pan YJ. Separation and characterization of synthetic impurities of triclabendazole by reversed-phase high performance liquid chromatography/electrospray ionization mass spectrometry. J Pharm Biomed Anal. 2005; 37(1): 97-107.