ANEW METHOD FOR ESTIMATION MEBENDAZOLE IN ITS PHARMACEUTICAL PREPARATIONS AND IN CAMEL URINE

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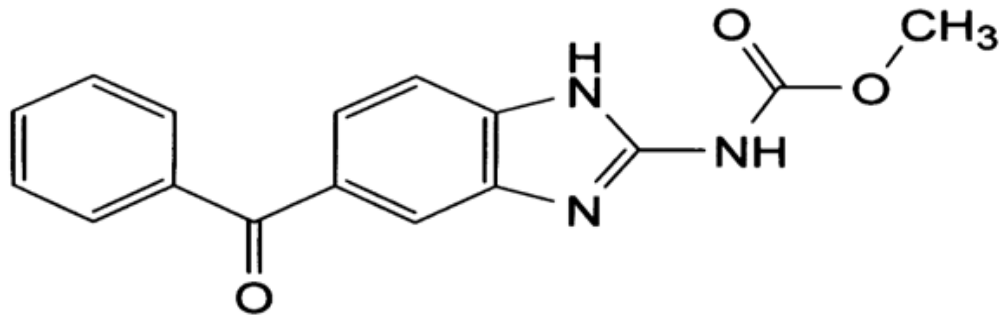
ABSTRACT

A simple, accurate and rapid spectrophotometric method has been developed for the estimation of mebendazole in pure, pharmaceutical formulations and camel urine. The method is based on the reaction of mebendazole with 1-fluoro 2, 4-dinitrobenzene to produce orange-yellow colored complex having maximum absorption at 494 nm. Bee's law was obeyed over the concentration range of 20-280µg/ml,with molar absorptivity of $3.32x10^4$ l/mol.cm. The present method is considered to be simple because it does not need heating, hydrolysis and solvent extraction steps. The proposed method has been successfully applied for the determination of mebendazole in pure ,pharmaceutical formulations and in camel urine.

INTRODUCTION

Mebendazole, chemically, Methyl-5-benzoyl-2-benzimidazole carbamate (Figure 1)[1], is a benzimidazole derivative that has been widely used in the treatment of hookworm, pinworm, roundworm, tapeworm, threadworm and mixed infestations. It is available in tablet and syrup form. Depending on the type of worm to be treated the dosage varies in adults and children. In Veterinary Medicine it is administered orally to horses at target dose of 8.8mg/kg and to sheep and goats at target dose of 15mg/kg [1-4]. Mebendazole is one of the most widely used drugs for the control of helminthes The drug is known to act through irreversible inhibition of glucose uptake in the parasite, leading to depletion of glycogen store.[5-7].



Methyl-(5–benzoyl–1*H*-benzimidazol–2–yl) carbamate

C16H13N3O3=295.3

Figure 1: chemical structure of Mebendazole .

Different analytical methods reported for the assay of mebendazole in various dosage forms include infrared spectroscopy [8], differential pulse polarography [9], fluorescence [10], HPLC methods [11,12],and spectrophotometric methods[13,14]. In addition to Liquid chromatographic methods [15], Voltammetric methods [16], volumetric titration method[17], and some UV-spectrophotometric methods [18-20]. The proposed method has been successfully applied for the determination of mebendazole in pure form, pharmaceutical preparations and camel urine.

MATERIAL AND METHODS

Apparatus

A spectra scan 50 UV-visible spectrophotometer with 1.0 cm quartz cells was used, [UVS-2700, Labomed, INC], USA.

Reagents

All chemicals used were of analytical grade and the standard material mebendazole was provided by state company of drug industries and medical appliance (SDI) Samara– Iraq.

Standard solution of Mebendazole (1 mg/ml):-

0.1 g of mebendazole was dissolved in 10 ml of 1% hydrochloric acid in ethanol, shaken to dissolve and the volume was adjusted to100 ml with ethanol in a volumetric flask.

1-fluoro 2,4-dinitrobenzene(1%):-

This solution was prepared by dissolve 1 g of 1-fluoro 2, 4-dinitrobenzene in 100ml ethanol in a volumetric flask.

Sodium hydroxide solution (0.1 N):-

This solution was prepared by dissolving 4 g of sodium hydroxide in 100 ml of distilled water in a volumetric flask.

Recommended procedure:

An aliquots of standard solution of Mebendazole (0.5-7.0 mg) were transferred into a series of 25ml volumetric flasks, 1 ml of 1N NaOH and 2 ml of 1-fluoro 2, 4-dinitrobenzene solution were added. The contents were diluted to the mark with distilled water. The absorbance was measured at 488 nm against a reagent blank.

Procedures for pharmaceutical preparations (tablets):

To minimize a possible variation in the composition of the tablets (100mg of Mebendazole /tablet)was used. Ten tablets were weighed , grounded, and powdered . Mebendazole (100mg) was then accurately weighed and added 10 ml of 1% hydrochloric acid in ethanol, shake for 30 minutes and diluted to 100.0 ml with ethanol and filtered using a whatman No.42 filter paper . Different volume of this solution was treated as mentioned under recommended procedure.

Procedures for pharmaceutical preparations (suspensions):

The contents of five bottles of suspensions.5ml of Vermosam suspension contain 100mg of Mebendazole) were mixed well. An aliquot corresponding to 100mg (5ml) of suspensions was diluted to 100 ml with ethanol in a volumetric flask. Different volume of this solution was treated as mentioned under recommended procedure.

Procedure for spiked camel urine: -

To demonstrate the practical applicability of the proposed method, 10 ml of camel urine were analyzed by this method. Camel urine was fortified with 1, 4 and 6 ml of 0.1% Mebendazole solution. The fortified camel urine samples were analyzed as described above under recommended procedure.

RESULTS AND DISCUSSION

The reaction of nucleophile substitution between mebendazole and 1-fluoro 2, 4-dinitrobenzene with the purpose of elaborating a new spectrophotometric method for the determination of mebendazole was investigated. The reaction occurred in slightly alkaline medium produces an orange-yellow color with maximum absorbance at 494 nm (Figure 2).

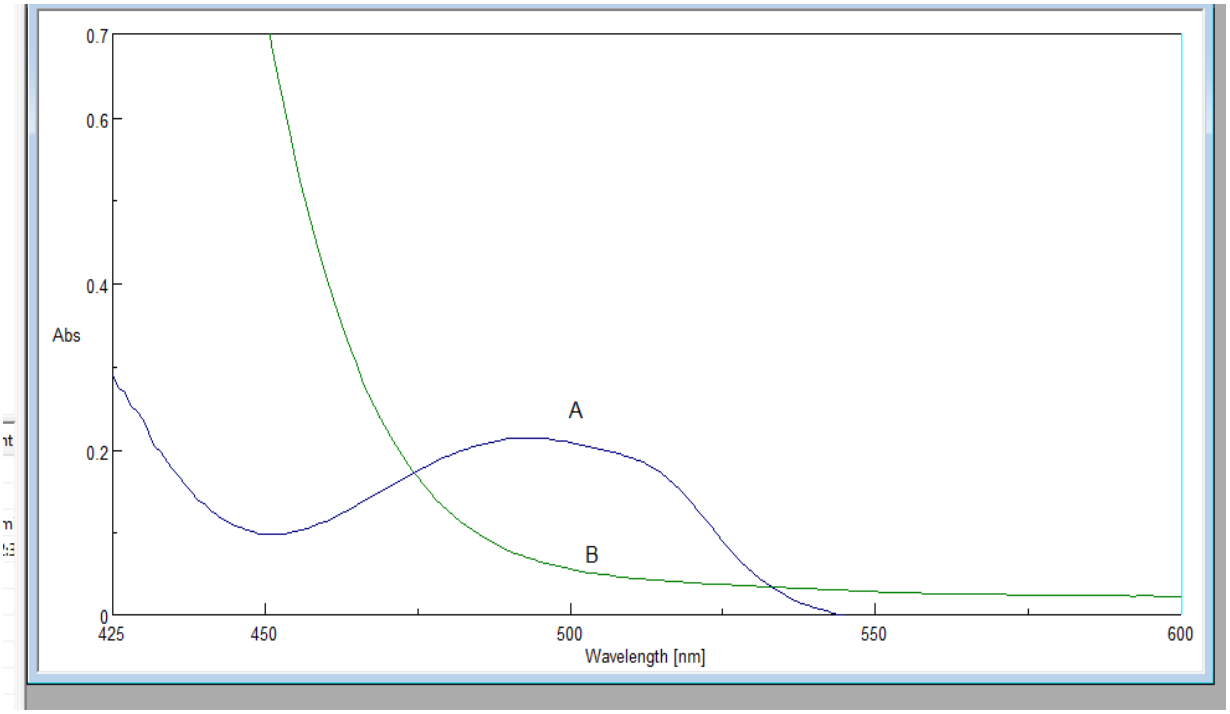


Figure 2: Absorption spectra of 3mg/25ml of mebendazole with 1-fluoro 2, 4-dinitrobenzene against reagent blank(A). Reagent blank against water (B).

The reaction variables were optimized by varying each variable and keeping others constant for obtaining maximum absorbance. The reaction was found to be quantitative in the sodium hydroxide medium. It was found that 1 ml of 1 N sodium hydroxide solution gives high sensitivity, 1-fluoro 2, 4-dinitrobenzene on the absorbance was investigated. A maximum and constant absorbance was founded with 1 to 2 ml of 1% of 1-fluoro 2, 4-dinitrobenzene solution and 2.0 ml has been used for subsequent experiments. The color reaction occurred at room temperature immediately and remained stable for at least 24h and a reaction time of 5 min was selected for reproducible results. Under the experimental conditions described, Beer's law is obeyed over the concentration range 20-280µg/ml (Figure 3).

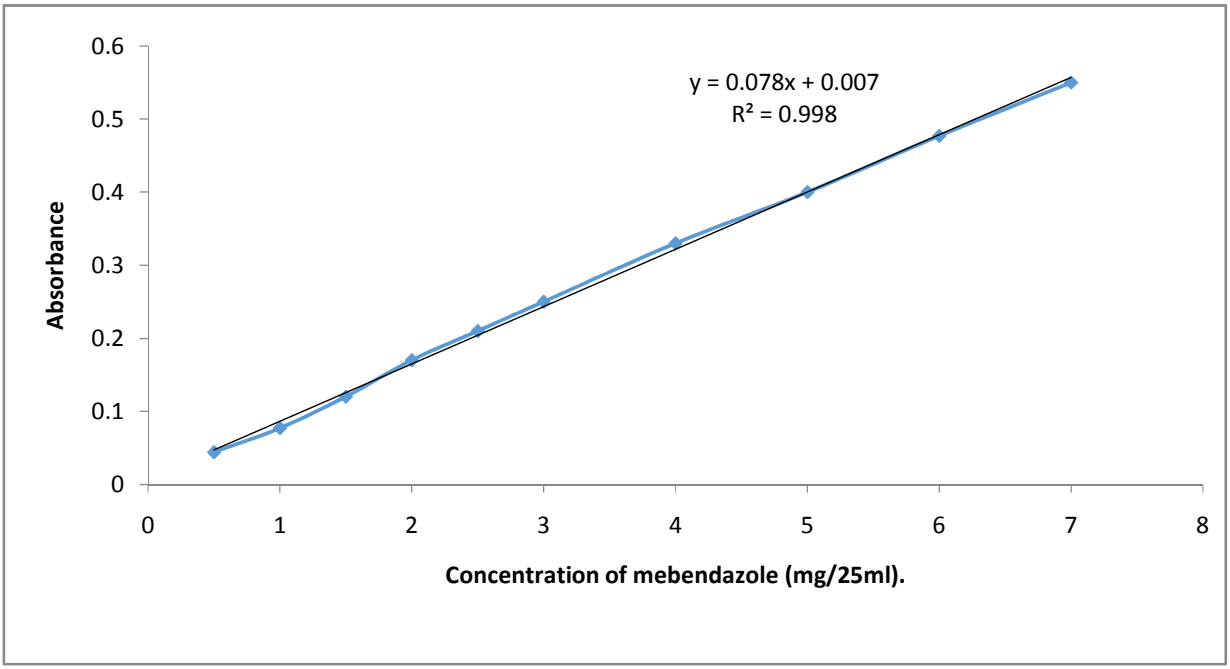


Figure 3: Calibration curve ofMebendazole.

A regression analysis of Beer's law plot at 488 nm revealed a good correlation (r=0.9999, n=7) .The graph of the absorbance versus the concentration of mebendazole showed a low intercept (-0.017) and slope (0.028) described by a regression equation Y = ax + b (where x is the concentration of mebendazole in µg /ml, Y is the absorbance, a is the slope and b is the intercept. The apparent molar absorptivity was 1.346×10^{4} l. mol^{-1} .cm^{-1}. The optical characteristics are given in (Table 1).

Table 1: Optical characteristics and statistical data for regression equation

Parameters	Value
λ max (nm)	488
Beer´s law limits (µg .ml^{-1})	20 – 280
Molar absorpitivity (1.mol^{-1}.cm^{-1})	3.32x10^{4}
Determination coefficient (r^2)	0.9987
Regression equation (Y= a × + b)	
Slope (a)	0.0785
Intercept (b)	0.0077

Accuracy and precision

The accuracy and precision of the method was established by analyzing the pure drug solution at three different levels. The average recovery which is a measure of accuracy is 100 ± 1.5 revealing high accuracy of the method. The relative standard deviation (RSD) , which is an indicator of precision is better than ±2% (Table 2).

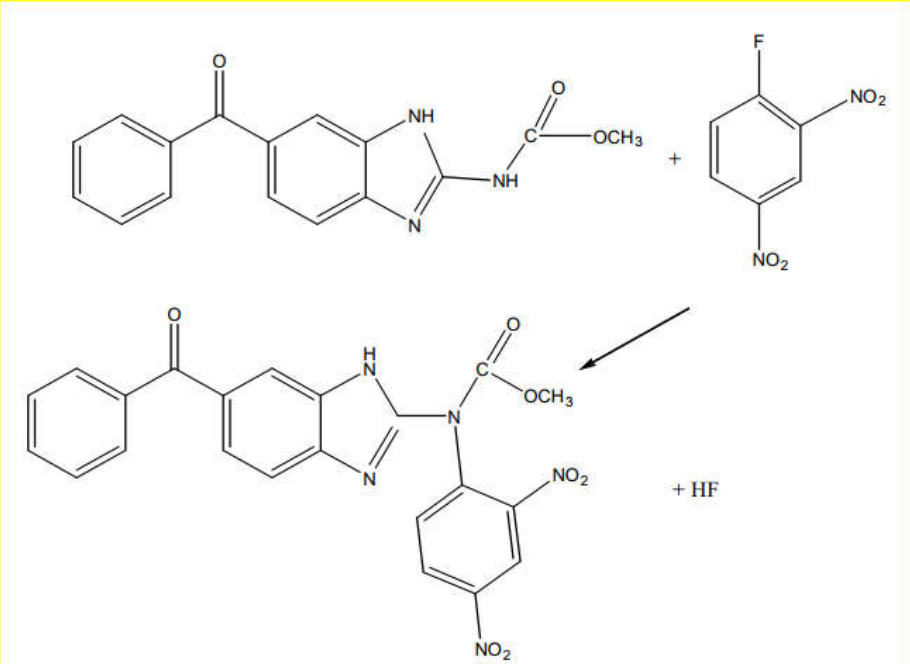
Table 2:Accuracy and precision of the proposed method.

Mebendazole taken µg/ml	Mebendazole found µg/ml	Er (%)[a]	RSD (%)
20	20.3	1.5	1.9
100	99.4	0.6	1.3
200	201.5	0.75	1.5

a: Mean of six determinations

Stoicheiometry of reaction

The stoicheiometry of the reaction between mebendazole and 1-fluoro 2, 4-dinitrobenzene was investigated using job's method of continuous variation and mole ratio methods of equal molar solution(3.386x10^{-3}M). The result obtained showed that (1:1) 1-fluoro 2, 4-dinitrobenzene to drug at 488 nm. The suggested reaction and structure of the product might be written as:



Apparent stability of the product

The conditional stability constant of the product can be estimated by using the following equation [21]

$K=a-(\Delta A/\varepsilon) /n^n (\Delta A/\varepsilon)^{n+1}$ Where: a= total mebendazole concentration. (Molar)

ΔA=Sample absorbance in reagent excess minus the sample absorbance at stiochiometric mount.

ε = Molar absorptivity at the measured wavelength and n=number of ligand .

The stability constant (means of three values) was found to be $1.48x10^7$ l/mol indicating the product is very stable.

The effect of foreign compounds or excipients in assay of mebendazole

The interfering effect of foreign species often accompanied with mebendazole in the pharmaceutical preparations were studied by adding different amounts of foreign species to 200µg/ml of mebendazole in solution .The recommended procedure for the determination of mebendazole was followed . The species are considered to interfere seriously if the cause aching of more than 2% in the absorbance obtained for mebendazole alone [22]. The results of the

recovery analysis are presented in (Table 3). Excipients at the concentration revealed do not interfere with the assay (Table 3).In addition recoveries in most cases were around 100%.

Table 3: Determination of mebendazole in presence of excipients

Excipients	Amount taken, (µg/ml)	Average recovery, * %
Talc	1000	100.10
Mannitol	1000	100.16
Mg – stearate	1000	100.12
Starch	1000	99.90
Microcrystalline cellulose	1000	99.98

* Average of three replicate determinations.

Application of the proposed method

The proposed method was successfully applied to the analysis of mebendazole in Vermosam tablets, Vermosam suspension, and spiked camel urine samples. The result of analysis for pharmaceutical formulations reveled that there is close agreement between the results obtained

by the proposed method and the label claim (Table 4), and the results of spiked camel urine samples. The recovery values obtained were close to100% (Table 5).

Table 4:Assay of mebendazole in pharmaceutical formulations.

Pharmaceutical formulation supplied by SDI	Amount of mebendazole* Proposed method	Label claim	%Recovery
Vermosam tablets 100mg/tablet	99.4 mg	100 mg	99.4
Vermosam suspension[each 5ml contain 100mg	100.1mg/5ml	100mg/5ml	100.1

*Mean of ten determinations.

Table 5:Determination of mebendazole in spiked camel urine

samples	mebendazole(µg/ml)*		%Recovery
	Taken	Found	
camel urine	40	٤٠	١٠٠
	100	99.5	99.5
	160	159	99.37

*Mean of ten determinations.

CONCLUSION

In the current study, a simple, rapid, precise and accurate spectrophotometric method was developed and validated for the determination of mebendazole in pharmaceutical preparations and spiked camel urine samples .The method free from such experimental variables as heating or solvent extraction step. The method rely on the use of simple and cheap chemicals and techniques and can be used for rapid routine determination and quality control of mebendazole in pure form, pharmaceutical preparations (tablets , suspension) and spiked camel urine sample.

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طريقة جديدة مبتكرة لتقديرِ الميبندازول في مستحضراتهِ الصيدلانيةِ وفي ادرارِ الجَملِ

نايف رحمن احمد ،فرحه خلف عمر

الخلاصة

تم اختبار طريقة طيفية جديدة تمتاز بالبساطة والدقة والسرعة لتقدير ميبندازول في حالته النقية وفي بعض مستحضراته الدوائية وفي ادرار الجمل، تعتمد الطريقة على تفاعل ميبندازول مع ١- فلور٢,٤- ثنائي نايترو بنزين لتعطي ناتج برتقالي-اصفراللون له أقصى امتصاص عند الطول ألموجي٤٩٤ نانوميتر وأمكن تقدير الكميات التي تتراوح بين٢٠-٢٨٠ مايكروغرام\مل وان معامل الامتصاص المولاري للطريقة المقترحة هو٣.٣٢×١٠^{٤} لتر .مول$^{-١}$.سم$^{-١}$. تتميز الطريقة بالبساطة والانتقائية والسرعة والدقة وتعتبر بسيطة كونها لاتحتاج للتسخين ولا للاستخلاص المذيبي . وتم دراسة الظروف المثلى للتفاعل .وطبقت الطريقة بنجاح لتقدير ميبندازول في حالته النقية وفي مستحضراته الصيدلانية وفي ادرار الجمل.

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