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Two simple methods for the estimation of albendazole and its dosage forms using chloramine-T

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Abstract

Two simple, rapid and reliable methods for the determination of albendazole are described. Both methods involve the use of chloramine-T as the oxidimetric reagent. In the titrimetric method, a known excess of chloramine-T is added to an acidified solution of sample, and after a specified time, the residual oxidant is determined iodometrically. Spectrophotometric procedure also involves the addition of a measured excess of chloramine-T in buffer medium of pH 2.70±0.1 and after the reaction is ensured to be complete, the surplus oxidant is determined by a well established colour reaction involving metol and primary arylamine that results in charge-transfer complex measurable at 520 nm. In both methods, the amount of chloramine-T corresponds to the drug content. Reaction conditions were examined and optimised. Titrimetry is based on a 1:3 stoichiometric reaction between albendazole and chloramine-T and is applicable in the range of 1–15 mg. In spectrophotometry, the absorbance was found to decrease linearly with increasing concentration of albendazole, which is corroborated by the calculated correlation coefficient value of −0.9998. The system obeys Beer's law for 2.5–25 μg ml^{-1} of albendazole. The molar absorptivity and Sandell sensitivity were calculated to be 6.24×10^3 l mol^{-1} cm^{-1} and 42.54 ng cm^{-2}, respectively. The limits of detection and quantification were calculated to be 1.15 and 3.83 μg ml^{-1}, respectively. The proposed methods were successfully applied to the determination of albendazole in commercially available dosage forms. The reliability of the assays was established by parallel determination by the official method and recovery studies.

Keywords: Albendazole; Determination; Titrimetry; Spectrophotometry; Chloramine-T; Charge-transfer complex; Dosage forms

1. Introduction

Albendazole, chemically known as methyl-5-(propylthio)-2-benzimidazole carbamate [1] is widely used as an anthelmentic having a wide spectrum of activity [2]. The therapeutic interest in this compound justifies research to establish analytical methods for the determination of albendazole in pharmaceutical preparations and in biological samples. Of the many methods available for monitoring albendazole therapy, high performance liquid chromatography (HPLC) offers the potential for the rapid analysis of the drug and its metabolites. Most of the reported methods require extensive sample preparations prior to chromatography and some apply only to the assay of drug in biological fluids [3,4], meat samples [5], veterinary formulations [6] and pure drug [7] and one or more metabolites in plasma [8–12,18]. Even the methods using the technique of liquid chromatography have been limited to the assay of the drug in milk [13,14], food products [15], cattle liver [16] and pork muscle tissue [17] and its metabolities Markus and Sherma have reported a gas chromatographic-mass spectrometric method for the determination of albendazole residues in cattle liver [19]. Differential scanning calorimetry and HPLC [20] have been applied to evaluate the compatibility of tablet excipients with albendazole. Only one procedure using HPLC has been described for the determination of albendazole in pharmaceuticals[21] but the procedure is poorly sensitive with the linear range being 0.1–0.4 mg ml^{-1}.

Two procedures based on ultraviolet spectrophotometry [22,23] are reported for the determination of the

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drug in formulations, but they are less sensitive and the technique is not very selective. Both direct and indirect visible spectrophotometric methods based on the colour reactions involving the amino or the propyl thio groups are also found in the literature. Zarapkar and Deshpande [24] have determined the drug in tablets and syrup based on the reduction of Folin–Ciocalteu reagent to a blue coloured species by the amino group of the drug molecule and subsequent measurement of absorbance at 700 nm. An extractive spectrophotometric method [25] using three acid dyes, bromophenol red, bromophenol blue and bromothymol blue has been reported for the assay of 2–12 μg ml^{-1} of drug in tablets and syrup. Recently, two indirect spectrophotometric procedures [26] involving *N*-bromosuccinimide–metol–sulphanilamide and permanganate celestine blue have been proposed by Sastry et al. Though the visible spectrophotometric procedures are fairly sensitive, they suffer from one or the other disadvantage like poor selectivity [24], extraction step [25] and use of unstable reagent solutions [26]. To our knowledge, no titrimetric method for the determination of albendazole in pharmaceuticals has yet been reported inspite of the versatility of the technique in chemical analysis.

In this paper, two methods are proposed for the determination of albendazole in bulk drug and in tablets based on its oxidation reaction with chloramine-T. Both methods involve the addition of a measured excess of chloramine-T to the sample solution followed by the determination of the unreacted oxidant either by iodometric titration or by spectrophotometry based on a colour reaction involving chloramine-T, metol and sulphanilic acid.

2. Experimental

2.1. Apparatus

A Systronics model 106 digital spectrophotometer with 1-cm matched glass cells was used for absorbance measurements.

2.2. Reagents

All chemicals used were of analytical reagent grade and distilled water was used to prepare reagent and sample solutions.

2.2.1. Chloramine-T

A 0.02 mol l^{-1} solution was prepared by dissolving about 5.6338 g of the oxidant in water and diluting to one litre with water. The solution was stored in an amber coloured bottle and standardised iodometrically[27] and used for titrimetric work. It was diluted appropriately with water to obtain a concentration of 500 μg ml^{-1} for spectrophotometric work.

2.2.2. Sodium thiosulphate

An approximately 0.04 mol l^{-1} solution was prepared by dissolving 4.88 g of the salt in water and diluting to one litre with water and standardised using pure potassium iodate [28].

2.2.3. Hydrochloric acid

A 5 mol l^{-1} acid was prepared by diluting 885 ml concentrated acid, Sp. gr.1.18 to 2 l with water.

2.2.4. Starch indicator (1%)

A paste of 1.0 g of soluble starch with a little water was poured, with constant stirring, into 100 ml of boiling water, boiled for a min and cooled.

2.2.5. Buffer solution

A mixture of 50 ml of 1 mol l^{-1} sodium acetate and 49.9 ml of 1 mol l^{-1} hydrochloric acid was diluted to 250 ml with water (pH 2.71).

2.2.6. Metol solution

A 0.2% solution was prepared by dissolving 200 mg of the reagent in 100 ml of water and stored in an amber coloured bottle. Fresh solution of the reagent was employed.

2.2.7. Sulphanilic acid solution (0.1%)

This was prepared by dissolving the 100 mg of the reagent in 100 ml of water and stored in an amber coloured bottle. Fresh solution of the reagent was employed.

2.2.8. Potassium iodide

A 10% solution of the reagent was prepared in water in the usual way.

2.2.9. Albendazole and its formulaitons

Pharmaceutical grade albendazole was kindly gifted by Cipla India Ltd., Mumbai, India and was used as received. Stock standard solution containing 2 mg ml^{-1} of albendazole was prepared by dissolving 500 mg of pure drug (accurately weighed) in 25 ml glacial acetic acid and diluting to 250 ml in a graduated flask. The solution was diluted stepwise to a working concentration of 100 μg ml^{-1} for spectrophotometric assay.

2.3. Procedures

2.3.1. Titrimetry

A 10 ml aliquot of the standard solution containing 1–15 mg of albendazole was accurately measured into a glass-stoppered Erlenmeyer flask and acidified by adding 5 ml of 5 mol l^{-1} hydrochloric acid. Ten milliliters

of 0.02 mol l^{-1} chloramine-T solution were added to the flask by means of a pipette and the contents were mixed well and set aside for 5 min with occasional shaking. Then, 10 ml of 10% potassium iodide solution were added, and the liberated iodine was titrated with 0.04 mol l^{-1} sodium thiosulphate solution using starch indicator towards the end-point. The experiment was repeated without albendazole solution. The amount of the drug in the aliquot was calculated from:

$$\text{mg of drug} = \frac{(B - S)\ M_w\ R}{N}$$

where B is the volume of thiosulphate consumed in the blank titration; S is the volume of thiosulphate consumed in the sample titration; M_w is the relative molecular mass of the drug; R is the strength of thiosulphate solution in mol ml^{-1}; and N is the number of moles of chloramine-T required to react with each mole of albendazole.

2.3.2. Spectrophotometry

In each of a series of 10 ml calibrated flasks were placed 0.0–2.5 ml of 100 μg ml^{-1} albendazole solution by means of a micro burette. Then, 2 ml of the buffer solution were added to each flask followed by 1.5 ml of 500 μg ml^{-1} chloramine-T solution. The contents were mixed well and the flasks were let stand for 10 min with occasional shaking. Finally, 1 ml each of 0.2% metol and 0.1% sulphanilic acid solutions were added to each flask and the volume was diluted to the mark with water. The absorbance of each solution was measured at 520 nm against a water blank after 10 min. A calibration graph was prepared by plotting the absorbance versus concentration of albendazole or a regression equation was deduced. The concentration of the unknown was read from the calibration graph or calculated using the regression equation.

2.3.3. Assay procedure for dosage forms

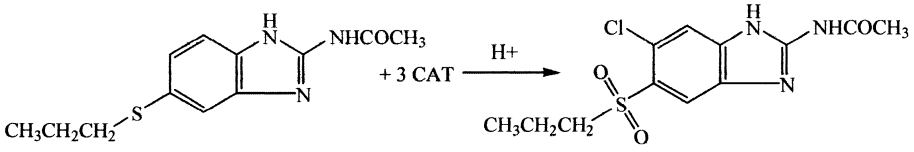
Dosage forms containing albendazole were purchased from local commercial sources. Twenty tablets were weighed accurately and ground into a fine powder. The amount of the powder equivalent to 200 mg of active component was accurately weighed into a 100 ml calibrated flask, 10 ml of glacial acetic acid and 50 ml of water were added, and shaken thoroughly for about 20 min. Then, the volume was diluted to the mark with water, mixed well and filtered using a Whatman no. 41 filter paper. First 10 ml portion of the filtrate was rejected and a convenient aliquot was assayed by titrimetry. The filtrate (2 mg ml^{-1}) was appropriately diluted to 100 μg ml^{-1} solution and the steps described under spectrophotometry were applied on a convenient aliquot.

3. Results and discussion

Chloramine-T is a valuable reagent for the titrimetric [29–35] and spectrophotometric [36–42] determination of many organic compounds of therapeutic interest. Its use depends mainly on its ability to cause oxidation of diverse functional groups. The present work is based on the oxidation of the sulphur atom of the propyl thio group of the albendazole molecule by a measured excess of chloramine-T and subsequent determination of the unreacted oxidant either by titrimetry or spectrophotometry.

3.1. Titrimetry

Chloramine-T reacts readily and quantitatively with albendazole in hydrochloric acid medium with the formation of chloro albendazole sulphone as shown below:



(CAT : Chloramine-T)

The reaction proceeds at room temperature and the stoichiometry of the reaction is 1:3 with respect to albendazole and chloramine-T.

In order to establish a back-titration method, it was necessary to consider several factors. Therefore, the effect of reaction time, the excess of chlormaine-T required and the hydrochloric acid concentration were investigated. The oxidation reaction was found to be complete in 5 min and standing times upto 30 min had no effect on the stoichiometry of the reaction. Three-fold excess of chlormaine-T had no significant effect on the stoichiometry of the reaction and the results were

reproducible for reaction times of 5–30 min. Even the hydrochloric acid concentration was not critical. Identical molar-ratio was obtained when 2–10 ml of 5 mol l^{-1} hydrochloric acid in a total volume of 25 ml was used. The relationship between the drug amount and the end-point was ascertained by calculating the correlation coefficient via the method of least squares and the value was found to be −0.9998 suggesting a definite stoichiometric reaction in the range (1–15 mg) investigated.

3.2. Spectrophotometry

In mildly acidic conditions, when compounds containing primary aromatic amino groups are made to react with 4-*N*-methylamino phenol (metol) and chloramine-T, a purple–red colour [43] is produced. In the present work, the above observation was used to determine micro quantities of chloramine-T which formed the basis for the indirect spectrophotometric determination of albendazole. In the proposed method, albendazole was treated with a known excess of chloramine-T and the unreacted chloramine-T was determined by metol–sulphanilic acid (primary arylamine) as a chromogenic agent.

Albendazole, when added in increasing amounts to a fixed amount of chloramine-T, consumes the oxidant proportionately, and consequently there will be concomitant fall in the chloramine-T concentration. This is indicated by the proportional decrease in the absorbance of the chromogen when fixed amounts of metol and sulphanilic acid are used. This decrease in absorbance was found to be proportional to albendazole concentration (Fig. 1).

In a preliminary experiment, different amounts of chloramine-T were reacted with specified amounts (as indicated in the procedure) of metol and sulphanilic acid in acetate–hydrochloric acid buffer, colour developed was measured at 520 nm, to determine the upper limit of chloramine-T that could be measured using the proposed colour reaction. The system was found to obey Beer's law upto 900 μg of chloramine-T. Hence, in the determination of albendazole, varying amounts of the drug were reacted with fixed amounts chloramine-T (750 μg), metol (1 ml of 0.2%) and sulphanilic acid (1 ml of 0.1%) throughout at pH 2.71 ± 0.10 in a total volume of 10 ml.

The reaction between albendazole and chloramine-T is fast and complete in 5 min, and standing times upto 20 min before the addition of metol and sulphanilic acid had no effect on the absorbance of the coloured species. Full colour development took 10 min and was stable for 30 min.

Two blanks were prepared for this study. The reagent blank, which contained optimum concentration of all the reactants except albendazole, gave maximum absorbance. The other blank was prepared in the absence of the drug and chloramine-T to determine the contribution of other reagent to the absorbance of the system. Since the second blank showed negligible absorbance, the absorbance of the developed colour was measured against water blank. The decreasing absorbance values at 520 nm were plotted against increasing concentration of albendazole to obtain the calibration graph.

Beer's law was obeyed over the range, 2.5–25 μg ml^{-1} albendazole. Using the method of least-squares, the regression equation describing the calibration graph was $A = 0.6562 \pm 0.023C$, where A is the absorbance and C

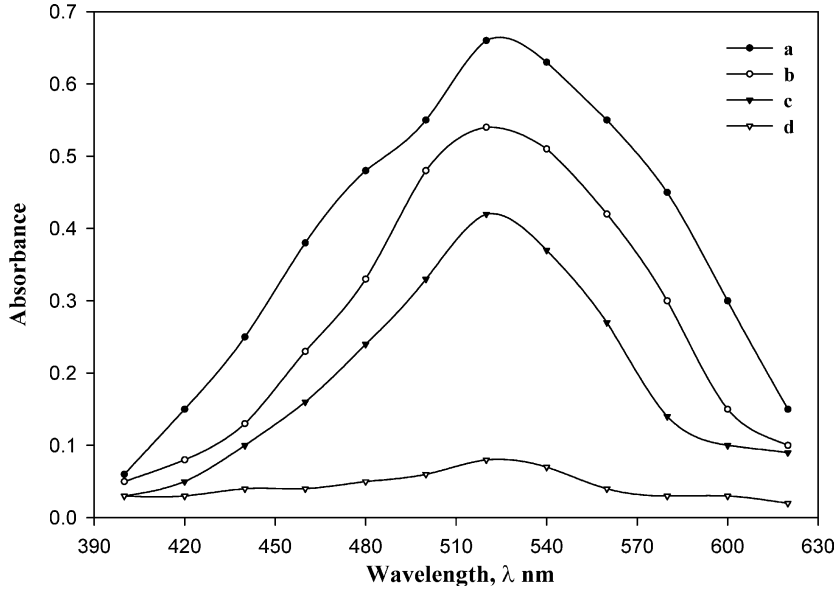


Fig. 1. Absorption spectra of the reaction product recorded against water. (a) Reagent blank. After treating with (b) 5, (c) 10 and (d) 25 μg ml^{-1} of the drug, respectively.

Table 1
Accuracy and precision

Titrimetry					Spectrophotometry				
Amount of drug taken (mg)	Amount of drug found [a] (mg)	Error (%)	SD	RSD (%)	Amount of drug taken (μg)	Amount of drug found [a] (μg)	Error (%)	SD	RSD (%)
4.00	4.01	0.25	0.02	0.50	50.00	50.77	1.54	0.31	0.63
8.00	7.93	0.88	0.17	2.10	100.00	99.43	0.57	1.24	1.25
12.00	12.21	1.33	0.07	0.58	150.00	149.76	0.16	0.53	0.36

[a] Average of seven determinations.

Table 2
Between-day precision

Titrimetry				Spectrophotometry			
Amount of drug taken (mg)	Amount of drug found [a] (mg)	Error (%)	RSD (%)	Amount of drug taken (μg)	Amount of drug found [a] (μg)	Error (%)	RSD (%)
3.00	3.06	2.00	0.40	125.00	124.73	0.22	0.62
7.00	7.05	0.71	1.98	175.00	175.35	0.20	0.73
10.00	10.06	0.60	0.97	200.00	202.67	1.34	0.73

[a] Mean value of five determinations performed over a period of 5 days.

concentration of albendazole in μg ml^{-1}. The correlation coefficient was −0.9998 ($n = 6$). The calculated values of molar absorptivity and Sandell sensitivity were found to be 6.24×10^3 l mol^{-1} cm^{-1} and 42.54 ng cm^{-2}, respectively. The limits of detection and quantification were 1.15 and 3.83 μg ml^{-1}, respectively.

The chemistry of the colour reaction may be suggested on the basis of a previously reported mechanism [44–49]. It is believed that the *p*-*N*-methyl benzoquinone monoimine formed in situ from the metol–chloramine-T combination, being a good electron acceptor, forms the charge-transfer complex with the electron donor, sulphanilic acid.

3.3. Accuracy and precision

Accuracy and precision of the proposed methods were evaluated by performing seven replicate analyses on the standard solution at three different levels. The accuracy defined in terms of percentage deviation of the calculated amount from the actual is listed in Table 1. Within-day precision expressed in terms of coefficient of variation (CV) is also given in Table 1. Between-day precision was similarly evaluated and presented in Table 2. Within-day CVs varied from 0.36 to 2.10% and between day CVs from 0.40 to 1.98% as can be seen from Tables 1 and 2, respectively. The values, respec-

Table 3
Results of assay of albendazole in tablet preparations

Tablet brand name	Label claim mg/tablet	Found [f] (% recovery ± SD)			Student's *t*-value[g]		*F*-value [h]	
		Titrimetry (T)	Spectrophotometry (S)	Reference method	T	S	T	S
Alminth [a]	200	101.88 ± 1.90	101.67 ± 0.81	100.98 ± 0.91	1.01	1.27	4.46	1.26
	400	98.84 ± 1.21	97.92 ± 1.59	99.32 ± 1.10	0.66	1.65	1.21	2.09
Albazole [b]	400	101.10 ± 1.89	102.81 ± 2.01	101.01 ± 1.83	0.08	1.48	1.07	1.21
Dispel [c]	400	99.98 ± 1.21	98.36 ± 1.52	98.81 ± 1.33	1.46	0.50	1.21	1.31
Nopar [d]	400	97.65 ± 1.34	98.76 ± 1.32	97.45 ± 1.46	0.23	1.51	1.14	1.17
Panamint [e]	400	101.81 ± 1.31	101.21 ± 1.49	100.31 ± 1.29	1.82	1.02	1.03	1.33

[a] Marketed by Torrent.
[b] Marketed by Wings Pharma.
[c] Marketed by Indchemie.
[d] Marketed by Malladi Drugs.
[e] Marketed by Seagull.
[f] Average of five determinations
[g] Tabulated value at 95% confidence level is 2.77.
[h] Tabulated value at 95% confidence level in 6.39.

tively, indicate the high precision and ruggedness of the proposed methods.

3.4. Application

The proposed methods were applied to the assay of albendazole in tablet preparations available in the local market and the results are presented in Table 3. The same batch tablet preparations were also analysed by the official method [50]. Statistical analysis of the results of analysis of tablets by the proposed and official methods using Student's t-test and F-test showed no significant difference with regard to accuracy and precision. The reliability and accuracy of the methods were further ascertained through recovery studies using the standard-addition technique. A fixed amount of albendazole from pre-analysed tablets was taken and pure (standard) albendazole at three different levels was added and the total was found by the proposed methods. Each level was repeated three times using three different market formulations. Percent recoveries of the added pure drug given in Table 4 indicate that the commonly encountered tablet excipients such as talc, starch, gum acacia, lactose, sodium alginate and magnesium stearate did not interfere in the determination by the proposed methods.

Although determination of albendazole has been studied extensively by various techniques, majority of them, especially chromatographic are devoted to either body fluids or drug metabolities. The only HPLC method [21] for the assay of the drug in pharmaceuticals lacks sensitivity. The proposed titrimetric method is simple, rapid, free from critical experimental variables and applicable over a wide range (1–15 mg). As small as 0.5 mg of the drug can be assayed with the loss of some accuracy. The spectrophotometric method developed is more sensitive than the UV spectrophotometric methods [22,23] but less sensitive than the visible-spectrophotometric method [26] employing permanganate–celestine blue as reagents ($\varepsilon = 3.66 \times 10^4$ l mol^{-1} cm^{-1}). The procedure is also twice as sensitive as the previously reported method based on a similar reaction but using N-bromosuccinimide as the oxidant [26] ($\varepsilon = 3.58 \times 10^3$ l mol^{-1} cm^{-1}). However, both procedures [26] employ oxidants which are unstable and require daily standardisation. An additional advantage of the proposed methods lies in the use of chloramine-T as the oxidimetric reagent which keeps its strength for several months if properly stored [27]. The methods can be used successfully for the determination of albendazole in tablet samples. While retaining the accuracy and precision of the other methods, the proposed methods are superior in terms of simplicity and convenience and are therefore suitable for routine analysis.

Table 4
Results of recovery studies by the standard-addition method

Tablets used	Titrimetry				Spectrophotometry				
	Amount of drug in formulation (mg)	Amount of pure drug added (mg)	Total found (mg)	% Recovery of pure drug [a]	Amount of drug in formulation (μg)	Amount of pure drug added (μg)	Total found (μg)	% Recovery of pure drug [a]
Alminth (200 mg)	1.02	4.00	5.00	99.50	50.83	50.00	101.21	100.76
	1.02	8.00	9.10	101.00	50.83	100.00	150.21	99.38
	1.02	12.00	13.21	101.58	50.83	150.00	201.08	100.17
Dispel (400 mg)	1.00	4.00	5.10	102.50	49.18	50.00	98.82	99.28
	1.00	8.00	9.21	102.63	49.18	100.00	149.63	100.45
	1.00	12.00	12.93	99.42	49.18	150.00	200.01	100.55
Nopar (400 mg)	0.98	4.00	4.90	98.00	49.38	50.00	100.00	101.24
	0.98	8.00	9.11	101.63	49.68	100.00	150.31	100.93
	0.98	12.00	13.03	100.42	49.38	150.00	200.89	101.01

[a] Mean value of three determinations.

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