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N-Bromosuccinimide as an analytical reagent for the Spectrophotometric determination of benzimidazole anthelmintics

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Two simple spectrophotometric methods are described for the determination of albendazole, mebendazole or fenbendazole based on oxidation of the drug with a known excess of N-bromosuccinimide (NBS) and estimating the unreacted NBS with metol-sulphanilamide (λmax 520 nm; Method A) or celestine blue (λmax 540 nm; Method B).

ALBENDAZOLE (ABZ), mebendazole (MBZ) and fenbendazole (FBZ) chemically known as Methyl 5-(Propyl thio)-2-benzimidazole carbamate, Methyl 5-benzoyl-2-benzimidazole carbamate and Methyl 5-(phenyl thio)-2-benzimidazole carbamate respectively are widely used as anthelmintics having a wide spectrum of activity. ABZ (USP[1]) and MBZ (USP[1] and IP[2]) are official while FBZ is not official. A review of literature revealed that spectrophotometric (ABZ[3-5], MBZ[6-9], FBZ[10-11]), Fluorimetric (MBZ[9, 12-13]), titrimetric (ABZ[14], MBZ[15]) and HPLC (ABZ[16-17], MBZ[18-19], FBZ[20- 21]), methods were reported.

In the present communication, the development of two visible spectrophotometric methods and their application for routine assay of ABZ, MBZ, or FBZ in pharmaceutical dosage forms have been described. These methods are based on the oxidation of ABZ, MBZ or FBZ by NBS followed by the estimation of reacted NBS (originally taken - unreacted) which corresponds to drug concentration either with metol-sulphanilamide (method A) or with celestine blue (azine dye, C.I. No. 51050, method B). The coloured species formed in method A is a charge - Transfer complex between sulphanilamideand P-N-methyl benzoquinone mono imine (*in situ* oxidation product from metol and NBS[22]). The reduction in colour intensity in method B is due to oxidation of CB with NBS giving a mixture of products from the dye with rupture of the conjugate system[23].

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MATERIALS AND METHODS

Aqueous solutions of NBS (Loba: 880 μg/ml for method A; 100 μg/ml for method B), metol (Loba, 0.3%), sulphanilamide (IDPL, 0.2%), glacial acetic acid (Ranbaxy), HCl (E. Merk; 5N) and celestine blue (100 μg) were prepared in the usual way.

Working standard solutions of ABZ, MBZ or FBZ (100 μg ml) method A; 10 μg/ml, method B) were prepared in 1:1 aqueous acetic acid. Spectrophotometer model 106 (systronics and Milton Roy 1201 spectronic were used for optical measurements.

Method A

Aliquots of standard solution representing 100-500 μg of (ABZ or FBZ) or 125-500 μg of (MBZ), 1 ml of NBS (880 μg/ml) and adequate volume of water to make 10 ml were successively added to a

Table I
Optical characteristics of ABZ, MBZ and FBZ using proposed methods

parameters	Method A			Method B		
	ABZ	MBZ	FBZ	ABZ	MBZ	FBZ
Beer's law limits (μg/ml)	4-20	5-25	4-20	0.4-2.0	0.8-2.4	0.4-2.0
Molar absorptivity ($lmole^{-1}$ cm^{-1})	$3.58x10^3$	$2.95x10^3$	$4.19x10^3$	$3.66x10^4$	$2.48x10^4$	$3.97x10^4$
Sandell's sensitivity ($\mu g/cm^2$ 0.001 abs unit)	0.074	0.10	0.071	0.007	0.012	0.008
Regression equation (Y*)						
Slope (b)	$1.32x10^{-2}$	$9.90x10^{-3}$	$1.40x10^{-2}$	$1.38x10^{-1}$	$8.28x10^{-2}$	$1.31x10^{-1}$
Intercept (a)	$1.9x10^{-3}$	$1.0x10^{-3}$	$1.0x10^{-3}$	$-1.5x10^{-3}$	$2.4x10^{-3}$	$6x10^{-4}$
Correlation coefficient(r)	0.9999	0.9999	0.9998	0.9998	0.9997	0.9997
Relative standard deviation (%)**	1.4	1.0	0.75	0.72	1.4	0.90
% Range of error (0.05 level)	1.48	1.06	0.80	0.76	1.48	0.95

* Y = a+bc; where C is concentration in μg/ml and Y is absorbance unit
** Six replicate samples.

series of 25 ml graduated test tubes. The contents of each tube were mixed well and kept aside for 15 min. Then 1.0 ml of metol solution and after 2 min, 2.0 ml of sulphanilamide were added and the volume in each tube was made upto the mark with distilled water. The absorbances were measured against distilled water at 520 nm during the stability period of 10-30 min. A blank experiment was also carried out omitting the drug. The descrease in the absorbance corresponding to ABZ, MBZ or FBZ was obtained by subtracting the absorbance of the drug solution from that of the blank. The amount of the drug was computed from the clibration curve similarly prepared.

Method B

Into a series of 25 ml graduated test tubes containing 10-60 μg of (ABZ, MBZ or FBZ), 1.25 ml of 5 M HCl and 2.5 ml of NBS (100 μg/ml) were added and the total volume in each tube was made upto 15 ml with distilled water and kept aside for 10 min. Ten ml of CB was added and mixed throughly and the absorbances were measured after 5 min at 540 nm against distilled water. Blanks were suitably prepared omitting the drug. The absorbance corresponding to consumed NBS, which in turn corresponds to drug concentration was obtained by substracting the absorbance of the blank solution from that of the test solution. The amount of the drug was computed from calibration curve.

Analysis of Pharmaceutical Formulations

Tablets : An amount of the powdered tablets equivalent to 100 mg of (ABZ, MBZ or FBZ) was transferred into a 100 ml volumetric flask. About 50 ml of Glacial acetic acid was added and then made upto 100 ml with distilled water and filtered. Stock solution was further diluted to make working solutions (100 μg/ml and 10 μg/ml) with (1:1) aqueous acetic acid.

Syrup : Syrup was thoroughly shaken and volume equivalent to 100 mg of (ABZ or MBZ) was

Table II

Analysis of Pharmaceutical formulations of (ABZ, MBZ or FBZ) by reference methods

Pharmaceutical formulations	Labelled amount (mg)	Amount found@(mg)			% Recovery#	
		Method A	Method B	Reference*	Method A	Method B
1) ABZ:						
Tablet-I	400	399.14±5.03	399.77±4.51	398.86±2.57	99.48±1.21	99.38±1.11
Tablet-II	200	1.99.17±2.36	199.90±2.89	199.07±1.59	99.12±1.10	99.08±0.92
Syrup-I	100	99.94±0.85	99.49±1.57	99.56±1.06	99.48±0.91	99.28±0.98
Syrup-II	100	99.80±1.00	100.35±0.73	100.2±0.45	99.18±0.88	99.29±1.01
2) MBZ:						
Tablet-I	100	99.93±0.86	99.62±0.85	99.71±0.52	99.62±1.02	99.52-1.09
Tablet-II	100	99.82±0.94	99.64±1.72	99.56±0.84	99.40±1.02	99.34±1.19
Syrup I	100	100.05±0.85	99.87±0.95	100.03±0.61	99.56±1.04	99.67±1.02
Syrup-II	100	100.26±0.80	100.12±0.95	100.31±0.60	99.60±1.07	99.68±1.04
3) FBZ:						
Tablet-I	150	149.89±1.76	150.73±1.88	149.14±1.24	99.72±1.18	99.82±1.08
Tablet-II	150	150.19±2.01	149.46±1.76	149.78±1.74	99.80±1.08	99.49±1.18
Tablet-III	150	150.24±1.14	149.99±1.47	150.18±0.95	99.70±1.20	99.50±1.09
Tablet-IV	150	150.30±0.91	150.29±1.11	150.56±0.98	99.60±1.2	99.64±1.02

@ Average ± standard deviation of 6 determinations

* Reference methods (ABZ[5], MBZ[7] & FBZ[10])

Recovery of 50mg added to the pre analysed pharmaceutical formulations (average of three determinations).

transferred into a 100 ml volumetric flask containing 50 ml of glacial acetic acid, shaken well and diluted to 100 ml with distilled water. Stock solution was further diluted to make working solutions (100 μg/ml and 10 μg/ml) with (1:1) aqueous acetic acid.

The above working solution of tablets and syrup were analysed using the procedures of method A and B. The amount of (ABZ, MBZ or FBZ) present in the sample was computed from the clibration curve.

RESULTS AND DISCUSSION

Beer's law limits, Molar extinction coefficient, sandell's sensitivity, correlation coefficient, slope and intercept of the regression analysis using least square method, precision and accuracy of the analysis of six separate samples containing 3/4 of the amount of upper Beer's law limit in each method were summarized in Table 1. Recovery experiments were performed using the standard addition method, results are shown in Table 2. The values obtained by the proposed and reference methods (ABZ[5], MBZ[7], FBZ[10]) for the estimation of ABZ, MBZ or FBZ in pharmaceutical dosage forms were compared in Table 2.

NBS provides molecular bromine at low concentration in polar media. This reacts with the drug (ABZ, MBZ or FBZ) resulting in either oxidation, substitution or addition depending on the functional group present in the drug (probably a mixture of products is formed but it may be reproducible in

specified experimental conditions). The excess NBS can be estimated based on the (1:2) charge - transfer complex between sulphanilamide and p-N-Methyl benzoquinone mono imine (*in situ* oxidation product from metol and NBS) in method A. The remaining molecular bromine is involved in bromination of the dye (CB) to form colourless product in method B. The stoichiometry of the reaction between NBS and drug under the experimental conditions for both the methods has been found to be 1:1.35, 1:1 or 1:1.75 for ABZ, MBZ or FBZ respectively.

All the diluents, excipients and colouring matter that are usually present in the dosage forms (tablets and syrup) did not interfere in the proposed methods. The results indicate that the proposed methods are sensitive (B>A), accurate, precise and reproducible and can be used as an alternatives to the existing methods.

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