ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
26 (2001) 919–927

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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High-performance liquid chromatographic separation and determination of the process related impurities of mebendazole, fenbendazole and albendazole in bulk drugs

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Received 28 August 2000; received in revised form 9 April 2001; accepted 25 April 2001

Abstract

Simple, specific and rapid reversed-phase high-performance liquid chromatographic methods to separate and determine potential impurities of anthelmintic drugs viz., mebendazole, fenbendazole and albendazole are reported. These methods afforded efficient separation, good resolution and identification of all the impurities examined. The methods were successfully applied not only for quality assurance, but also process development of the select anthelmintic drugs. © 2001 Published by Elsevier Science B.V.

Keywords: Anthelmintic; Mebendazole; Fenbendazole; Albendazole; Process impurities

1. Introduction

Human infections caused by parasitic worms, represent one of the most important health problems in the world [1] and have a very important economic impact. Over the last two decades, substantial progress has been made in the discovery and development of drugs for the treatment of most of human infections. Benzimidazoles are a large family of such anthelmintic drugs having a broad spectrum of activity against nimotode parasites of the intestinal track. These anthelmentic drugs include mebendazole, fenbendazole and albendazole, which are well tolerated in animals. Recently, these drugs have been proved to be useful chemotherapeutic agents in the management of hydatid disease in man, especially in cases where the cyst is in-accessible or spillage has occurred during its removal by surgery [2]. Since these drugs are intended for oral consumption, they must be characterized as completely as possible. It is necessary that their purity and safety be ensured thoroughly before using them in different formulations. Their quality depends not only the procedures adopted but also the materials used in the manufacturing process. Synthetic precursors, side reaction products, unreacted raw materials, intermediates and products of degradation are likely impurities of these products. These impuri-

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0731-7085/01/$ - see front matter
PII: S0731-7085(01)00446-0

ties often posses unwanted toxicological effects due to which any benefit from their administration is outweighed. Therefore, a close monitoring of such impurities is of great importance in controlling the quality within the manufacturing environment. A number of analytical methods are reported in the literature for determination of the residues of anthelmintic drugs in milk [3,4], cheese [5], pork [6], meat [7], blood [8], liver [9], human plasma [10,11] and eel muscle tissue [12]. These methods mainly confined to the extraction and quantitative determination of the parent compounds and their metabolites in biological matrices. The official USP [13] and EP [14] have adopted methods which are based on the weak basicity of the imidazole nucleus enabling these drugs to be titrated nonaqueously against perchloric acid. But these methods are found to be neither specific nor accurate and suffer from the interference of potential impurities of related substances. Other methods for assaying these drugs include the use of UV–Visible spectrophotometry [15–20], polarography [21] and NMR techniques [22]. However, these are quite tedious and time consuming. A thorough literature search has revealed that HPLC [23–26] is widely used for routine analysis of anthelmintics in bulk drugs. However, these HPLC methods do not respond to the presence of impurities originating from known synthetic procedures. In this paper, a few simple, specific and rapid reversed-phase HPLC methods, not only for assay of mebendazole, fenbendazole and albendazole, but also for separation and determination of small amounts of their process impurities has been described.

Table 1
Retention and response data for mebendazole and potential impurities

Compound	Abbreviation	t_R (min)	RRT	N	T_f	RRF	RSD (%)	λ_{max} (nm)
2-amino-5-benzoyl-benzimidazole	ABB	3.95	0.21	2161	1.22	2.53	1.65	272
Methylbenzimidazole carbamate	MBC	5.06	0.27	3546	1.19	5.07	1.14	280
2-hydroxy-5-benzoyl-benzimidazole	HBB	7.89	0.42	8622	1.15	1.73	1.46	271
2-methyl-5-benzoyl benzimidazole	MBB	11.69	0.63	8412	1.12	4.11	1.85	263
Mebendazole	MEB	18.57	1.00	7642	0.82	1.00	1.33	292

$n = 3$.

Table 2
Retention and response data for fenbendazole and potential impurities

Compound	Abbreviation	t_R (min)	RRT	N	T_f	RRF	RSD (%)	λ_{max} (nm)
Methylbenzimidazole carbamate	MBC	3.54	0.46	1735	1.13	1.28	1.51	281
5-thiophenyl-2-methyl benzimidazole	TMB	4.23	0.55	2478	1.19	1.29	1.05	277
5-thiophenly benzimidazole	TPB	5.56	0.72	1903	1.15	1.00	1.43	268
Fenbendazole	FEN	7.67	1.00	8148	0.95	2.30	1.19	290

$n = 3$.

Table 3
Retention and response data for albendazole and potential impurities

Compound	Abbreviation	t_R (min)	RRT	N	T_f	RRF	RSD (%)	λ_{max} (nm)
Albendazole sulphoxide	ADE	8.91	0.36	10 995	0.92	1.87	1.05	256
Albendazole sulphone	ANE	11.70	0.47	18 959	1.13	1.78	1.43	242
Albendazole	ALB	25.08	1.00	87 117	0.94	1.00	1.19	235

$n = 3$.

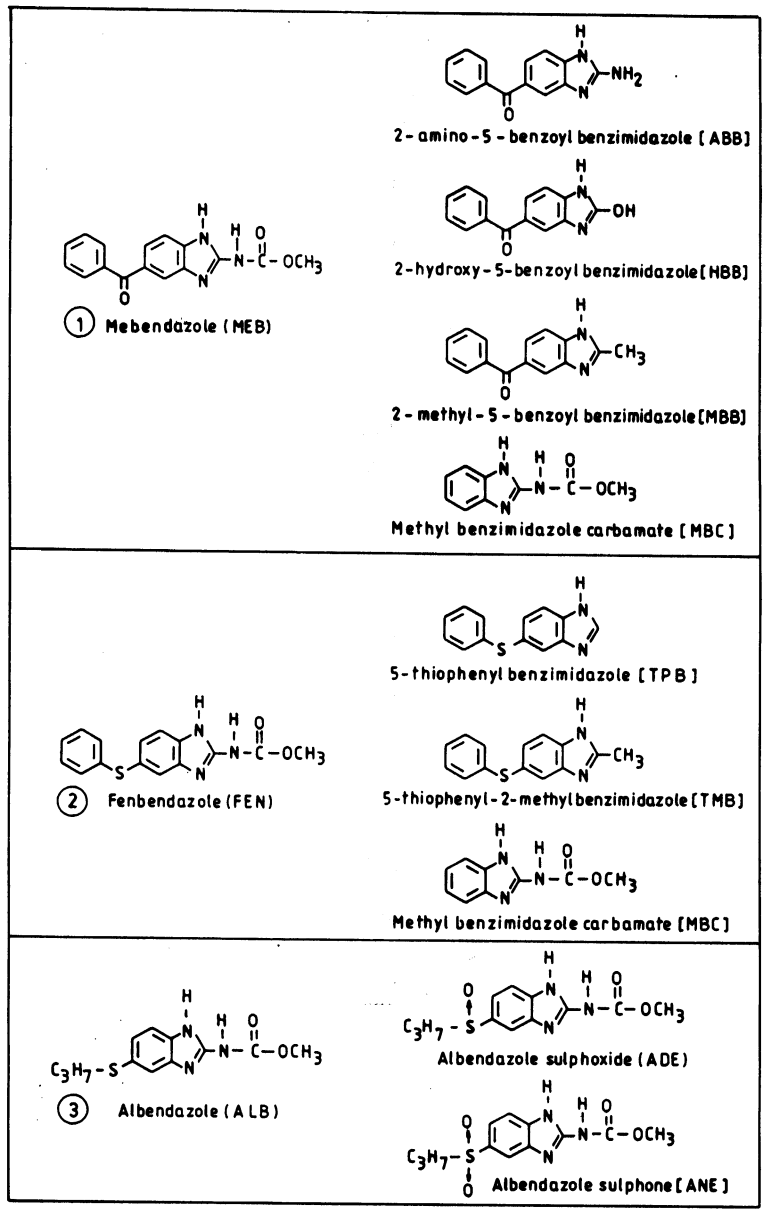

Fig. 1. Chemical structures of anthelmintics, mebendazole (1), fenbendazole (2), albendazole (3) and their process impurities.

2. Experimental

2.1. Materials and reagents

Analytical reagent grade orthophosphoric acid, formic acid, perchloric acid (E. Merck, Mumbai, India), potassium hydroxide, hydrochloric acid (SD Fine Chem, Mumbai, India) were used. HPLC grade acetonitrile, methanol and acetic acid were obtained from Qualigens, Mumbai, India. De-ionized water and samples of mebendazole, fenbendazole, albendazole and their impurities as free bases were prepared in our R & D laboratory.

2.2. Apparatus

A high-performance liquid chromatograph (Waters, Milford. M.A, USA), model 510 pump with a 20 μl loop injector having a six-way high pressure valve was used. A Waters model 486,

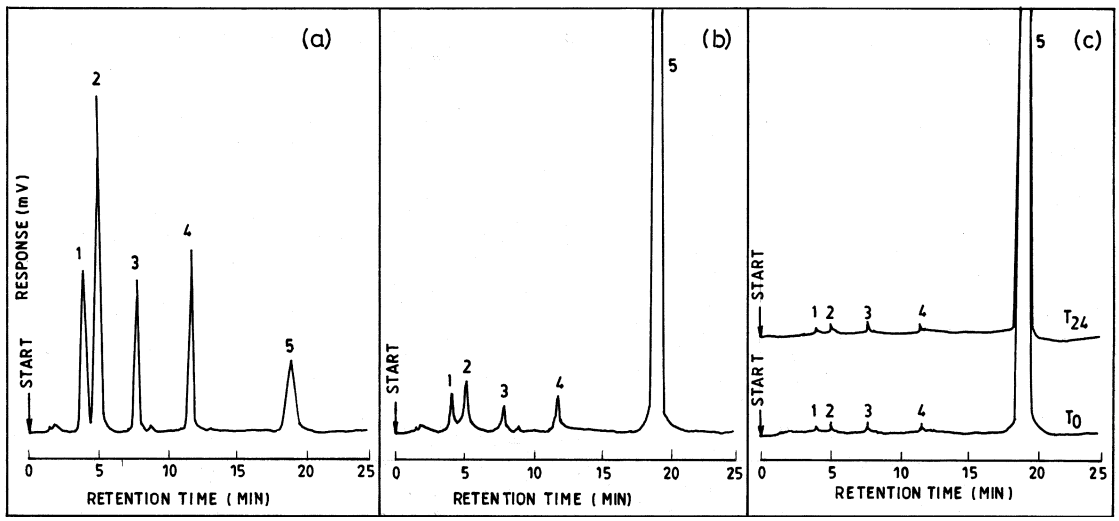

Fig. 2. Typical chromatograph of: (a) a mixture containing: (1) ABB (2 μg); (2) MBC (2 μg); (3) HBB (2 μg); (4) MBB (2 μg); (5) MEB (5 μg). (b) MEB (50 μg) spiked with low level impurities containing (1) ABB (0.025 μg); (2) MBC (0.025 μg); (3) HBB (0.025 μg); and (4) MBB (0.025 μg). (c) A finished product of MEB (50 μg) stored in the mobile phase at ambient conditions for T_0 0 h and T_{24} 24 h.

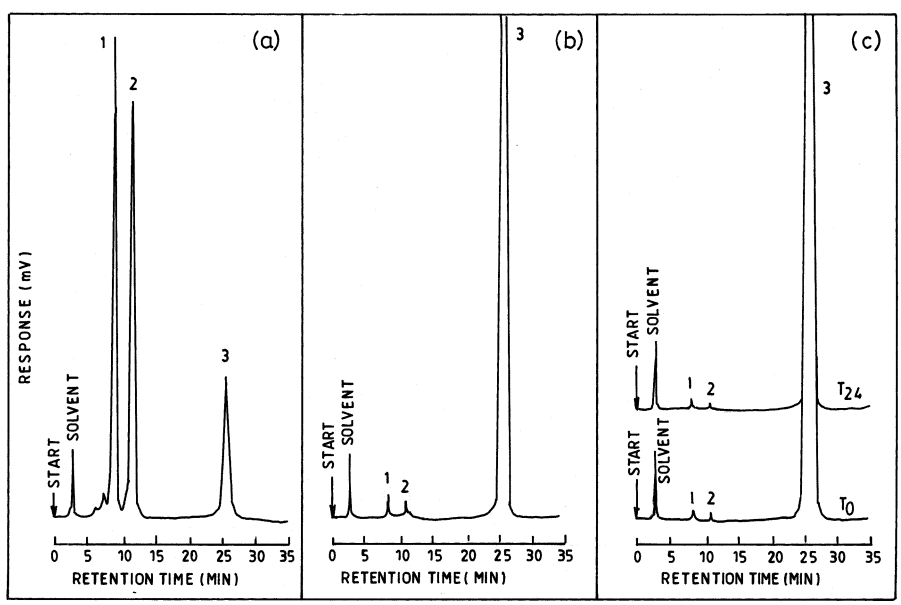

Fig. 3. Typical chromatograph of: (a) a mixture containing: (1) MBC (4 μg); (2) TMB (4 μg); (3) TPB (4 μg); (4) FEN (4 μg). (b) FEN (40 μg) spiked with low level impurities containing (1) MBC (0.04 μg); (2) TMB (0.04 μg); (3) TPB (0.04 μg). (c) A finished product of FEN (40 μg) stored in the mobile phase at ambient conditions for (T_0) 0 h and (T_{24}) 24 h.

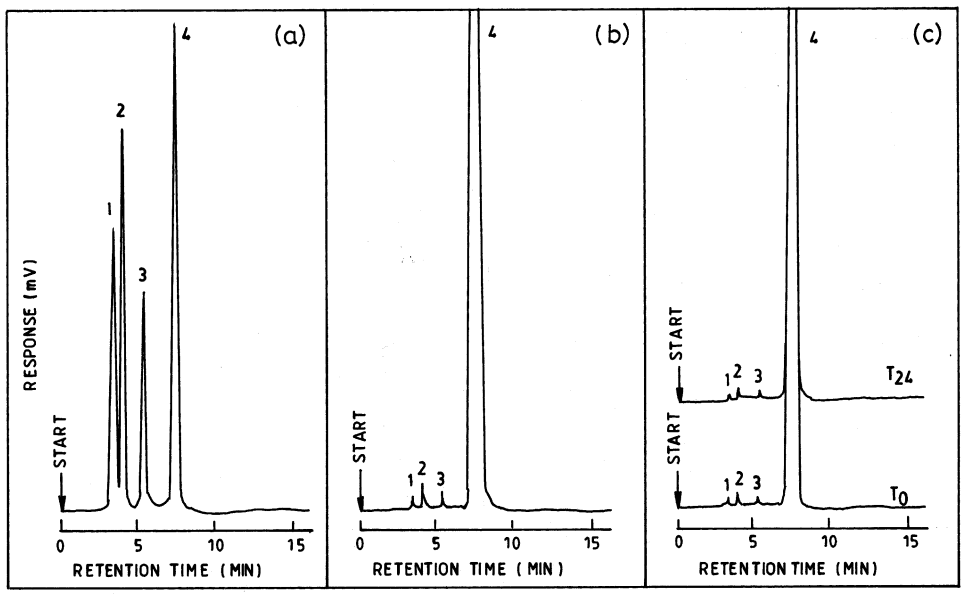

Fig. 4. Typical chromatograph of: (a) a mixture containing: (1) ADE (8 μg); (2) ANE (8 μg); (3) ALB (8 μg). (b) ALB (80 μg) spiked with low level impurities containing: (1) ADE (0.04 μg); (2) ANE (0.04 μg). (c) A finished product of ALB (80 μg) stored in the mobile phase at ambient conditions for 0 h (T_0) and 24 h (T_{24}).

tunable wavelength UV–Visible spectrophotometric detector was connected after the column. A reversed-phase Inertsil C_{18} (GL Sciences Inc., Tokyo, Japan) column (250 × 4.6 mm ID; particle size 5 μm; pore size 100 A^0) was used for the separation of the three products and their impurities. The chromatographic and integrated data were recorded with Waters 745 B Data module. The pH measurements were carried out with digital pH meter (Elico, Hyderabad, India) model LI120, equipped with a combined glass–calomel electrode, which was calibrated using standard buffer solutions of pH 4.0, 7.0, and 9.2 before measuring the pH of the solutions.

2.3. Chromatographic conditions

The mobile phase for mebendazole was a mixture of orthophosporic acid (0.1% v/v in H_2O) and acetonitrile (70:30 v/v), pH adjusted to 6.0 with 1 M potassium hydroxide solution, whereas for fenbendazole the mobile phase was orthophosporic acid (0.1% v/v in H_2O) and acetonitrile (40:60 v/v), pH adjusted to 4.0 with 1M potassium hydroxide solution. Methanol–water–perchloric acid (45:55:0.3 v/v/v) was used as mobile phase for albendazole. Before delivering into the system, the mobile phase was passed through 0.45 μm, PTFE filter and degassed using vacuum. The analysis was carried out under isocratic conditions at a flow rate of 1 ml/min at room temper-

Table 4
Analytical data for standard mixtures

Compound	Taken (%)	Found (%)	Error (%)
ABB	0.78	0.80	2.56
MBC	1.62	1.67	3.09
HBB	0.87	0.89	2.30
MBB	0.95	0.98	3.16
MEB	95.78	95.66	0.13
MBC	1.22	1.26	3.28
TMB	2.54	2.63	3.54
TPB	0.68	0.70	2.94
FEN	95.56	95.41	0.16
ADE	2.45	2.52	2.86
ANE	2.28	2.35	3.07
ALB	95.27	95.13	1.05

$n = 3$.

Table 5
Linearity data

S. No.	Compound	Mass range $\times 10^{-9}$ g	Linear regression	Correlation coefficient	Limit of detection $\times 10^{-9}$ g
1	ABB	0.25–2.5	6.071 e6x − 22 754.58	0.986	0.06
2	MBC	0.60–3.2	11.634 e6x − 10 148.64	0.991	0.09
3	HBB	0.20–0.24	3.984 e6x − 4216.29	0.995	0.05
4	MBB	0.30–4.30	9.389 e6x − 9964.88	0.979	0.08
5	MBC	0.40–2.00	6.030 e6x − 19 202.59	0.983	0.09
6	TMB	0.70–3.00	6.055 e6x − 25 142.05	0.976	0.04
7	TPB	0.20–2.00	4.383 e6x + 656.27	0.997	0.07
8	ADE	0.30–4.00	7.904 e6x + 4515.49	0.987	0.08
9	ANE	0.40–4.00	7.830 e6x + 1232.00	0.994	0.08
10	MEB	200–800	2.413 e6x − 531 557.80	0.984	8.00
11	FEN	250–600	10.137 e6x + 100 765.00	0.990	3.00
12	ALB	500–1100	4.636 e6x − 136 926.00	0.998	5.00

ature (25 ± 2 °C) and a chart speed of 5 mm per minute. Chromatograms were recorded at their respective absorption maxima (λ_{max}) i.e. 290 nm for both mebendazole and fenbendazole, 254 nm for albendazole using a UV detector. The λ_{max} of the bulk drugs and process impurities were given in Table 1 Table 2 Table 3. The pH of the mobile phase was determined from the pK_a values of the respective drugs, which were determined in water, water/acetonitrile, water/methanol and found to be in the range of 5.5–7.5

2.4. Analytical procedure

Samples of mebendazole (0.25 mg/ml), fenbendazole (0.20 mg/ml) and albendazole (0.5 mg/ml) were prepared in the respective mobile phases. A 20 μl volume of each sample was injected and chromatographed under the above conditions. The amounts of impurities were calculated from their respective peak areas.

3. Results and discussion

Fig. 1 gives the chemical structures of the three anthelmintic drugs: (1) mebendazole; (2) fenbendazole; (3) albendazole and their potential process impurities examined in the present study. The drugs were characterized against reference standards of USP/EP and found to be chemically equivalent, where as the impurities were characterized thoroughly by NMR, IR, MS and the purity was checked by TLC.

3.1. Mebendazole (MEB)

Mebendazole, (5-benzoyl-1H-bezimidazole-2-yl) carbamicacid methyl ester is a broad spectrum anthelmintic drug widely used for the treatment of *Ascaris*, *Enterobius*, *Trichuris*, *Ancyclostoma*, *Ne-*

Table 6
HPLC determination of potential process impurities in the bulk drug of: (1) mebendazole; (2) fenbendazole; and (3) albendazole

Compound	Impurity	Concentration (%)	RSD (%)
Mebendazole	MBC	0.19	1.63
	HBB	0.02	1.27
	ABB	Nil	–
	MBB	Nil	–
Fenbendazole	MBC	0.15	1.48
	TMB	0.03	1.75
	TPB	Nil	–
Albendazole	ADE	0.23	1.39
	ANE	0.11	1.53

$n = 3$.

cator, *Trichinella*, *Strongyloids*, *Dipylidiam*, *Hymenolepis Taenia* and all parasites affecting man [27]. It could be seen from Fig. 1 that, 2-amino-5-benzolylbenzimidazole (ABB), 2-methyl 1,5-benzoylbenzimidazole (MBB), methylbenzimidazole carbamate (MBC), 2-hydroxy-5-benzoylbenzimidazole (HBB) are the potential impurities of MEB. Fig. 2, shows the typical HPLC chromatogram of a synthetic mixture containing MEB and its process impurities. These are well separated by a reversed-phase C_{18} column with a mixture of orthophosporic acid (0.1% v/v in H_2O)/acetonitrile (70:30 v/v) adjusted to pH 6.0 ± 0.5 with 1 M potassium hydroxide solution as mobile phase. It may be noted that the pH of the mobile phase is very critical, a deviation from an apparent value of 6.0 resulted in a sharp shift in the retention time of MEB. However, a small change of ± 0.5 yields acceptable results as the shift in retention times of impurities as well as MEB is found to be negligible. For identification of peaks in the chromatogram, authentic samples were injected and the retention times were compared. Table 1, gives the retention time (t_R), relative retention time (RRT), theoretical plates (N) , tailing factor (T_f) and relative response factors (RRF), of MEB and its potential impurities. The relative standard deviations were calculated from the peak areas for an average of three injections.

3.2. Fenbendazole (FEN)

Fenbendazole, [5-(phenylthio)-1H-benzimidazole-2-yl] carbamic acid methylester is a highly effective broad spectrum anthelmintic of the benzimidazole class of drugs. It is generally used as an antiparasitic agent in domestic animals, fish and in man. Methyl-benzimidazolecarbamate(MBC), 5-thiophenylbenzimidazole (TPB), 5-thiophenly-2-methylbenz-imidazole(TMB) are potential process impurities of FEN. It is a weakly basic hydrophobic compound with appreciable solubility in polar organic solvents. In an earlier attempt by Dimitrios J. Fletouris et al. [28] octane sulphonate as ion-pair reagent in highly acidic conditions was used, but severe peak tailing was noticed in the chromatogram. Owing to these properties, orthophosporic acid (0.1% v/v in H_2O)/acetonitrile (60:40 v/v) pH adjusted to 4.0 with 1 M potassium hydroxide, mobile phase seemed to be a good choice for the separation of FEN from its process impurities. These are well separated from FEN and are shown in Fig. 3. The impurities are identified by injecting the individual impurities. Table 2 shows the t_R, RRT, N, T_f and RRF of FEN and potential impurities.

3.3. Albendazole (ALB)

Albendazole, [5(propylthio)-1H-benzimidazole-2-yl] carbamic acid methyl ester, is another broad spectrum anthelmintic drug, which belongs to the group of benzimidazoles. It has shown efficacy against all classes of helminths that commonly occur in animals and human beings. However, toxicological studies have shown that its metabolites, namely albendazole sulphoxide (ADE) and albendazole sulphone (ANE) are teratogenic. In this regard, a maximum limit of 100 ng/mg for these impurities has been proposed by regulatory agencies [29]. Owing to their polar characteristics, albendazole and its sulphoxide and sulphone metabolites are not easily amenable to analysis with a single chromatographic system. Using silica based C_{18} or poly (styrene–divinylbenzene) stationary phases several methods have been described in the literature. Using these methods, the parent compound could be readily chromatographed, but its sulphoxide and sulphone metabolites had almost identical retention times. Albendazole and its potential impurities ADE and ANE are well separated using a reversed-phase C_{18} column with methanol–water–perchloric acid (45:55:0.3 v/v/v) as mobile phase. Fig. 4 shows the HPLC chromatogram of ALB and its potential impurities. Table 3 shows the retention time t_R, RRT, N, T_f and RRF of ALB and its potential impurities.

Three different synthetic mixtures containing known amounts of: (i) ABB, MBC, HBB, MBB and MEB; (ii) MBC, TMB, TPB and FEN; and (iii) ADE, ANE and ALB were prepared and analyzed using the developed HPLC conditions. Small quantities of impurities were added to the respective synthetic mixtures and chromatographed to check that these additions were

accurately reflected in their peak areas. All estimations were carried out thrice and relative standard deviations (RSD) were calculated (Table 4). The signal-to-noise value was determined to be 4.0 for detection of impurities as low as 0.04×10^{-9}. It can be seen from Table 4 that the measured amounts agree well with actual values and the mean recovery of mebendazole, fenbendazole and albendazole from authentic samples was found to be 99.84 to $99.87 \pm 0.14\%$. The UV detector was set at 290 nm for both MEB and FEN and 254 nm for ALB and used for both detection and quantification. The wavelength were selected based on the absorption maxima (λ_{max}) of the drugs recorded for the UV spectra. Good linearity was found between the mass and integral response for each compound under examination. The high level (75–120%) linearity data of bulk drugs as well as low level (0.05–0.5%) linearity data of impurities were determined and recorded in Table 5. It gives linearity equation, mass range and correlation coefficient. At 0.001 A.U.F.S the limits of detection (LOD) were 8.0×10^{-9} for MEB, 3.0×10^{-9} for FEN and 5.0×10^{-9} for ALB. The LOD values for all the process impurities were determined and recorded in Table 5. Bulk drugs viz., MEB, FEN and ALB were spiked with respective low level impurities and chromatographed. The chromatograms were shown in Fig. 2b, Fig. 3b and Fig. 4b, respectively. To determine the stability of MEB, FEN and ALB in mobile phase, the drugs were stored in their respective mobile phases for 24 h and chromatographed on the next day. The chromatograms were shown in Fig. 2c, Fig. 3c and Fig. 4c, respectively. It could be seen from the chromatograms that no significant change was observed.

The proposed HPLC methods have been successfully adopted to determine potential impurities in bulk drugs of different batches of mebendazole, fenbendazole and albendazole. The results are recorded in Table 6. From these results it could be seen that the developed methods are simple and useful for monitoring the potential impurities of anthelmintic drugs.

Acknowledgements

The authors wish to thank the management of CIPLA LTD., for encouragement and support, special thanks to Dr V.G. Nayak, Corporate Quality Assurance Manager and A.K. Malhotra, Factory Manager, CIPLA LTD., for their valuable suggestions.

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