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COMMUNICATION

Simultaneous Determination of Metronidazole Benzoate, Methylparaben, and Propylparaben by High-Performance Liquid Chromatography

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ABSTRACT

The simultaneous determination of metronidazole benzoate (MB), methylparaben (MP), and propylparaben (PP) in an oral suspension formulation was developed using high-performance liquid chromatography (HPLC). The method was developed using a Novapak C_{18} (3.9 × 150 mm, 4 μm) column, methanol-water (50:50, v/v) as the mobile phase and an ultraviolet (UV) detector at 254 nm. The peak area response versus concentration was linear in a concentration range from 40 to 400 μg/ml of MB, 0.8 to 8.0 μg/ml of MP, and 0.2 to 2.0 μg of PP. The correlation coefficients were 0.9997 for MB, 0.9987 for MP, and 0.9983 for PP, with relative standard errors of 1.12%, 1.28%, and 1.67%, respectively.

INTRODUCTION

Metronidazole benzoate (MB), 2-(2-methyl-5nitro-1*H*-imidazol-1-yl)ethyl benzoate, is an antiprotozoal nitroimidazole derivative. It is effective against both intestinal and hepatic amebiasis. It has also found use in the treatment of other protozoal diseases, such as giardiasis and balantidiasis (1). Metronidazole manifests antibacterial activity against all anaerobic cocci and both anaerobic gram-negative bacilli, including *Bacteroides* species, and anaerobic spore-forming gram-positive bacilli (2). A preservative system is generally required for oral suspension or syrup formulations containing sugar. Since they are not the principle active ingredients in the product,

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they may be overlooked when it comes to testing. Preservatives such as sodium benzoate, sorbic acid, methylparaben (MP), and propylparaben (PP), have been used for many years. The formulator must be fully aware of the ingredients of the preservative system used in the product and the need for them to be analyzed to establish their effectiveness throughout the shelf life of the product (3).

There are some analytical procedures reported in the literature for the determination of the MB individually by a spectophotometric method (4) and a high-performance liquid chromatography (HPLC) procedure in formulations (5). A simultaneous determination of the MP and PP in pharmaceutical formulations is described in the presence of other components using HPLC (6–11), gas chromatography (12,13), and high-performance thin-layer chromatography (14).

The purpose of this study was to develop a simple, precise, accurate, sensitive, and selective HPLC method for the simultaneous determination of MB, MP, and PP in oral liquid formulations. The suitability of the method was evaluated on various accelerated stability studies and commercial samples.

EXPERIMENTAL

Apparatus

The chromatographic system consisted of a Waters (Milford, MA) HPLC system consisting of a 610 solvent delivery system, 600 system controller, 717 plus auto sampler and injector, reverse-phase Waters Novapak C_{18} column (3.9 × 150 mm, 4 μm) and Waters 996 photodiode array detector. The chromatograms were recorded on a personal computer using Waters Millennium Chromatographic software.

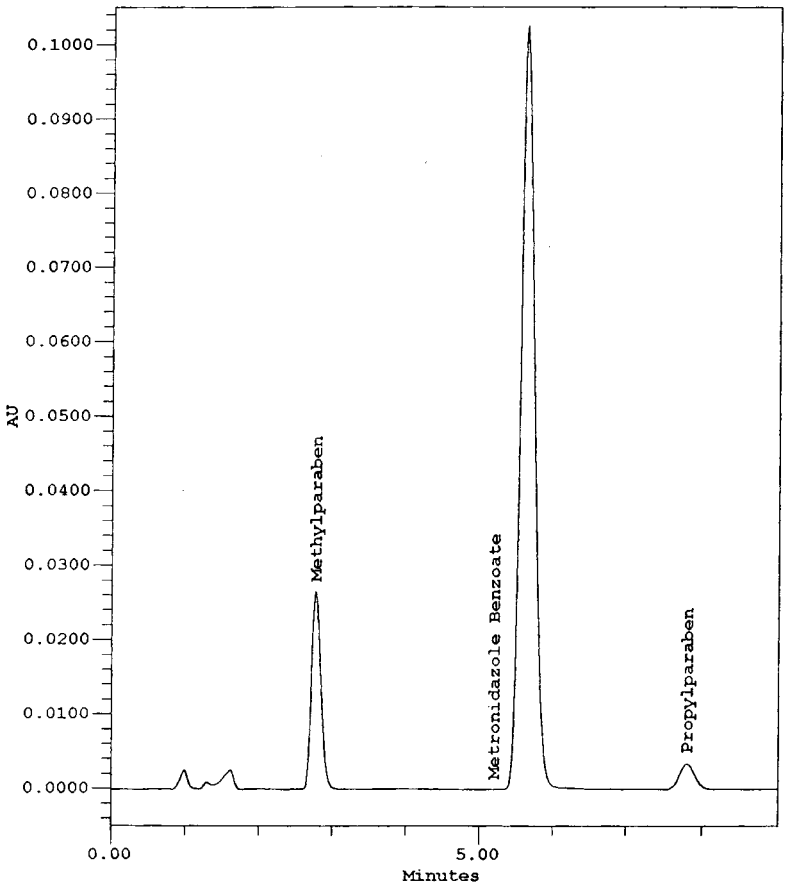

Figure 1. Chromatogram of test sample showing the separated peaks of methylparaben, metronidazole benzoate, and propylparaben.

Reference Substance, Reagents, and Chemicals

The MB was supplied by Fare Chemica s.r.l. (Italy); MP and PP of pharmaceutical grade were supplied by Nipa Laboratories (UK). Distilled water was obtained from a Milli-Q system (Millipore, Milford, MA). Solvents and chemicals (analytical grade) were obtained from Merck (Darmstadt, Germany). The MB reference standard was obtained from British Pharmacopoeia Commission Laboratories (Middlesex, UK). MP and PP reference standards were obtained from the U.S. Pharmacopeial Convention (Rockville, MD).

Chromatographic Conditions

The mobile phase used was a mixture of methanol and water (50:50 v/v). The analytical column was a Novapak C_{18} (3.9 × 150 mm, 4 μm) (Waters). All analyses were done under isocratic conditions at a flow rate of 1 ml/min and at room temperature. The ultraviolet (UV) detection wavelength was fixed at 254 nm. The volume of solution injected onto the column was 20 μl.

Samples

The test sample was a commercially available oral suspension with the following composition: 125 mg/5 ml of metronidazole, 4 mg/5 ml of MP, 1 mg/ml of PP, and an excipient quantity sufficient to produce 5 ml. Other test samples used were the accelerated stability samples with similar compositions.

Solution Preparation

Standard Solution

A standard stock solution of 2.0 mg/ml of MB, 0.04 mg/ml of MP, and 0.01 mg/ml of PP was prepared in

Table 1

Results Obtained in the Recovery Tests and Coefficients of Variation Using the HPLC Method

Sample	Theoretical Addition (mg)	Total Theoretical Amount (mg)	Determined Amount (mg)	Recovery[a] (%)	Coefficient of Variation (%)
Metronidazole benzoate					
A	2.47976	9.10398	9.05557	99.47	1.15
B	3.71964	10.34386	10.30659	99.64	0.56
C	6.19939	12.82361	12.82603	100.02	0.41
D	7.43927	14.06349	13.98990	99.48	0.61
E	9.91903	16.54325	16.44170	99.39	0.59
F	12.39879	19.02301	18.81229	98.89	0.78
Methylparaben					
A	0.08162	0.28234	0.28149	99.70	0.79
B	0.12244	0.32316	0.32358	100.13	0.40
C	0.20406	0.40478	0.40756	100.69	0.53
D	0.24487	0.44559	0.44663	100.23	0.65
E	0.32650	0.52722	0.52917	100.37	0.72
F	0.40081	0.60884	0.60891	100.01	0.79
Propylparaben					
A	0.02194	0.07057	0.07036	99.70	0.44
B	0.03290	0.08153	0.08142	99.87	0.67
C	0.05484	0.10347	0.10335	99.88	0.98
D	0.06581	0.11444	0.11391	99.54	0.75
E	0.08774	0.13637	0.13569	99.50	0.95
F	0.10968	0.15831	0.15651	98.87	0.54

[a]Average of six determinations.

methanol. A 5-ml sample of stock solution was diluted to 50 ml with methanol to produce concentrations of 200 μg/ml of MB, 4 μg/ml of MP, and 1 μg/ml of PP. The solution was filtered, and 20 μl was injected.

Test Solution

For the test solution, 5 ml of suspension was transferred to a 100-ml volumetric flask and diluted with methanol to volume; the solution was sonicated for 10 min and cooled to room temperature. The 5 ml were further diluted to 50 ml in a volumetric flask with methanol. The solution was filtered, and 20 μl was injected.

The final concentrations of MB, MP, and PP in the samples were calculated by comparison of sample and standard peak areas obtained with the average of three injections of standard solution.

RESULTS AND DISCUSSION

The wavelength of 254 nm was selected in order to permit the simultaneous determination of MB, MP, and PP in the oral suspension because MB is present in a much higher concentration than MP and PP and shows minimum absorption in this wavelength, whereas MP and PP present maximum absorption.

The mobile phase containing methanol-water allowed the elution of MB, MP, and PP with adequate retention time. The approximate retention time of each component was 3.0 for MP, 6.0 for MB, and 8.2 for PP (Fig. 1). The calibration curve was obtained in a concentration range from 40 to 400 μg/ml, 0.8 to 8.0 μg/ml, and 0.2 to 2.0 μg/ml of MB, MP, and PP, respectively. The regression curves were calculated by the least-squares method. The correlation coefficients were 0.9997 for MB, 0.9987 for MP, and 0.9983 for PP, and the relative standard errors of estimates were 1.12% for MB, 1.28% for MP, and 1.67% for PP.

The recovery tests were performed according to the methods of the Association of Official Analytical Chemists (AOAC) (15). MB, MP, and PP standard solutions were added to sample solutions, and the recovery data are presented in Table 1. The accuracy and precision of the proposed method was evaluated from the results obtained for each component in the oral suspension.

Accuracy was determined by obtaining the peak response of six different samples prepared from a single sample stock solution. The relative standard deviations were 0.078% for MB, 0.045% for MP, and 0.18% for PP. The precision was determined by analyzing the test sample by six replicate determinations.

A system suitability test according to USP was per-

Table 2

Results Obtained in the System Suitability Test Using HPLC

Sample	Component	Area	Peak Asymmetry	Theoretical Plates	Capacity Factor	Resolution	Selectivity
1	MP	490,872	1.141	1027	2.053	—	—
	MB	2,698,563	1.187	1848	5.387	7.084	2.623
	PP	96,148	1.101	3380	7.837	4.190	1.455
2	MP	490,660	1.173	996	2.047	—	—
	MB	2,699,301	1.215	1802	5.380	7.077	2.629
	PP	95,248	1.068	3528	7.847	4.215	1.458
3	MP	490,786	1.146	1020	2.050	—	—
	MB	2,698,857	1.192	1834	5.383	7.075	2.626
	PP	96,992	1.122	3268	7.833	4.173	1.455
4	MP	490,733	1.159	1012	2.048	—	—
	MB	2,699,106	1.198	1826	5.382	7.091	2.627
	PP	96,018	1.109	3348	7.832	4.191	1.455
5	MP	491,992	1.136	1031	2.053	—	—
	MB	2,704,641	1.182	1848	5.387	7.069	2.623
	PP	97,260	1.127	3255	7.837	4.167	1.455
6	MP	491,669	1.183	986	2.047	—	—
	MB	2,703,696	1.209	1805	5.380	7.071	2.629
	PP	96,533	1.086	3412	7.847	4.196	1.458

formed on the chromatograms obtained from standard and test solutions to check parameters like column efficiency, peak asymmetry, capacity factor, selectivity, and resolution. The results obtained from six replicate injections of the standard solution as representative chromatograms are summarized in the Table 2.

CONCLUSION

The proposed reverse-phase HPLC method is rapid, accurate, precise, sensitive, and selective for the simultaneous determination of MB, MP, and PP preservatives from the oral suspension formulation. Hence, the method can be applied for the routine analysis of the accelerated stability samples and the quality control of such suspensions containing these three ingredients.

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