Available online at www.ijpsdr.com
International Journal of Pharmaceutical Sciences and Drug Research 2014; 6(1): 70-74

Research Article ISSN 0975-248X

RP-HPLC Method Development and Validation for the Simultaneous Determination of Mebendazole and the Two Preservatives Methylparaben and Propylparaben in Pharmaceutical Oral Suspension Dosage Form

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ABSTRACT

A rapid, accurate, specific, linear, and sensitive reverse phase-HPLC method has been developed and validated for the simultaneous determination of Mebendazole (MEB), Methylparaben (MP) and Propylparaben (PP) in pharmaceutical oral suspension dosage form. The chromatographic separation was performed on (Inertsil ODS-3V) C_{18} Column (250mm × 4.6mm, 5µm particle size) using a mobile phase: Methanol, 0.05M monobasic potassium phosphate, Acetonitrile (48:32:20v/v), at a flow rate of 1.5 ml/min and 30°C column temperature with the detection wavelength at 247nm. The retention times of MP, MEB and PP were 2.83 min, 4.14 min and 4.75 min respectively. The linearity was performed in the concentration range of 3.6-5.4µg/ml (MP), 40-60µg/ml (MEB), and 0.4-0.6µg/ml (PP) with a squared correlation coefficient of 0.999, 0.999 and 0.9994 for MP, MEB and PP respectively. The percentage purity of MP, MEB and PP was found to be >99.0%. The Proposed method has been validated for specificity, linearity, precision, accuracy, ruggedness and robustness which were within the acceptance limit according to ICH guidelines and the developed method was successfully employed for routine quality control analysis in the combined pharmaceutical dosage forms.

Keywords: Methylparaben, Propylparaben, Mebendazole, RP-HPLC, Validation.

INTRODUCTION

Methylparaben [MP], methyl 4-hydroxybenzoate [Fig. 1] is a preservative. Its molecular weight is 152.156 g/mol with an empirical formula $C_8H_8O_3$. [1] Propylparaben [PP], Propyl 4-hydroxybenzoate [Fig. 2] is a preservative. Its molecular weight is 180.2 g/mol with an empirical formula $C_{10}H_{12}O_3$. [1] Both MP and PP are members of a group of chemical compounds known as Parabens which are commonly used as preservative in pharmaceutical, food and cosmetic products due to their anti-fungal and antibacterial properties. [2-5] The parabens are effective over a wide pH range. [3] The liquid preparations are particularly susceptible to microbial growth because of the nature of their ingredient. Such preparations are protected by the addition of preservatives that prevent the alteration and degradation of the products. [4] The assay method for parabens by using titration method described under methylparaben and propylparaben monographs of the united state pharmacopeia require a long time for sample

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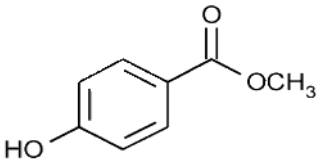

Fig. 1: Chemical Structure for Methylparaben

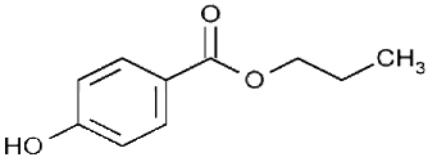

Fig. 2: Chemical Structure for Propylparaben

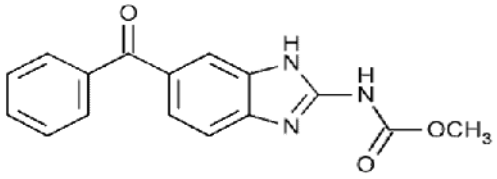

Fig. 3: Chemical Structure for Mebendazole

preparation. [1] RP-HPLC methods have been reported for the assay of parabens in cosmetic and pharmaceutical products. [6-9] Mebendazole [MEB], methyl(5-benzoyl-1H-benzimidazol-2yl)carbamate [Fig. 3]. Its molecular weight is 295.29 g/mol with empirical formula $C_{16}H_{13}N_3O_3$. [10]

Mebendazole is a very insoluble drug widely used for the treatment of intestinal helminthes infection. Literature survey revealed that only few HPLC methods for the analysis of mebendazole have been reported. [11] The united state pharmacopeia describes a method for the analysis of mebendazole in oral suspension by spectrophotometric method which is non specific in the presence of MP and PP as preservatives. [1] In the present work we are focused on to achieve the optimum chromatographic conditions for the simultaneous determination of MP, MEB and PP in the oral suspension. The developed method can be applied successfully to quality control purposes. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH guidelines [12-13], which is mandatory also.

MATERIALS AND METHODS

Instrumentation

Chemicals and Reagents

MP and PP were purchased from Merck and MEB Was purchased from API workshop. All chemicals used of HPLC grade: Acetonitrile and Methanol were purchased from J.T. Baker, and Formic acid was purchased from Merck. Water used was freshly prepared by Sama Pharmaceuticals Manufacturing Co.

Equipment

A Dionex UltiMate 3000 HPLC system with Chromeleon software "version 6.8", Photodiod Array Detector and Autosampler was used. It was manufactured by Dionex Corporation Company, USA.

Chromatographic Conditions

The column (Inertsil ODS-3V) C_{18} Column (250mm × 4.6mm, 5µm particle size) was used for analytical separation. The mobile phase consisted of mobile phase: Methanol, 0.05M monobasic potassium phosphate, Acetonitrile (48:32:20v/v). The flow was adjusted to 1.5 ml/min. The instrument was operated at 30°C temperature. The UV detection was achieved at 247nm and purity analysis was performed over a wavelength range of 200-400nm. The injection volume was 15µL.

Preparation of Analytical Solutions

Preparation of 0.05M Monobasic Potassium Phosphate

Prepared by dissolving 6.8 g of potassium dihydrogen phosphate in 1000 ml of distilled water.

Preparation of mobile phase

The mobile phase was prepared by mixing 480 ml of Methanol, 320 ml of 0.05M monobasic potassium phosphate and 200 ml of Acetonitrile. The pH was adjusted to 5.5 with 0.1M phosphoric acid or 1 M sodium hydroxide and degassed in ultrasonic water bath for 2 minutes. Filter through 0.45µ filter under vacuum filtration.

Preparation of mebendazole stock standard solution

The MEB standard stock solution was prepared by transferring MEB equivalent to 25.0 mg standard into 100 ml volumetric flask .10 ml of formic acid were added and heated at 50°C for 15 minutes then shook for 5 minutes. 90 ml of methanol were added, mixed and allowed to cool and the volume was completed with methanol and filtered using 0.45µ filter to obtain a solution having a concentration of 0.25 mg/ml.

Preparation of MP and PP stock standard solution

The standard was prepared by dissolving an equivalent to 45 mg of MP standard and equivalent to 5 mg of PP in 100 ml mobile phase, 5 ml of the resulting solution were diluted to 100 ml with mobile phase, mixed and filter through 0.45µ filter.

Preparation of MP, MEB and PP standard solution:

The standard solutions were prepared by diluting 5 ml of mebendazole stock standard solution and 5 ml of MP and PP stock standard solution to 25 ml in the same volumetric flask with mobile phase. Mixed and filter through 0.45µ filter.

Preparation of sample solution

The sample solution was prepared by transferring 25 ml of mebendazole oral suspension equivalent to 500 mg of mebendazole to 100 ml volumetric flask. 50ml of formic acid were added and heated in water bath at 50°C for 15 minutes, then shook by mechanical means for 1 hour, diluted with water to volume, mixed and filtered. 5ml of the resulting solution were transferred to 100 ml volumetric flask, and diluted to volume with a solution of formic acid (1:9). 5 ml of the resulting solution were diluted to 25ml with mobile phase. Pass the solution through a filter of 0.5µm filter.

Method Development and Validation of HPLC Method

The suggested analytical method was validated according to ICH guidelines with respect to certain parameters such as specificity, linearity, precision, accuracy, and system suitability.

Specificity

The specificity was carried out to determine whether there are any interference of any impurities (presence of components may be unexpected to present) in retention time of analytical peaks Forced degradation studies are carried out by using 3M HCl, 3M NaOH, thermal degradation Hydrogen peroxide and Photo degradation.

Linearity

Express ability to obtain test results where directly proportional to the concentration of analyte in the sample. The linearity of the method was established by a spiking a series of sample mixtures of MP, MEB and PP, the solutions of five different concentration levels 3.6-5.4µg/ml (MP), 40-60µg/ml (MEB) and 0.4-0.6µg/ml (PP) are injected into the HPLC system. Construct the calibration curves for the standard solutions by plotting their response ratios (ratios of the peak area of the analytes) against their respective concentrations linear regression was applied and slope-a, intercept-b, and correlation coefficient-R^2 were determined.

Precision

Express the closeness of agreement between the series of measurement obtained from multiple sampling of same homogeneous sample under the prescribed conditions.

Method precision was determined both in terms of repeatability (injection and analysis) and intermediate precision/Ruggedness (It shows the degree of reproducibility of test results obtained by analyzing the sample under variety of normal test conditions such as analyst, instruments).

In order to determine precision, six independent sample solution preparations from a single lot of formulation 4.5µg/ml for MP, 50µg/ml for MEB and 0.5µg/ml for PP were injected in to HPLC system, the retention time and peak area was determined and expressed as mean and %RSD

calculated from the data obtained which are found to be within the specified limits.

Accuracy

Accuracy was determined in terms of percentage recovery the accuracy study was performed for 80%, 100% and 120 % for MP, MEB and PP. Standard and sample solutions are injected into HPLC system in triplicate and percentage recoveries of MP, MEB and PP were calculated. The area of each level was used for calculation of % recovery.

Robustness

Robustness of the developed method was investigated by evaluating the influence of small deliberate variations in procedure variables like flow rate (± 6.6%), change in column temperature (± 5°C) and change in wave length (±5nm). The robustness was performed for the flow rate variations from 1.5ml/min to 1.6ml/min and 1.4ml/min and the method is robust even by change in the mobile phase B ±5%.

System suitability

System suitability test was carried out on freshly prepared standard solution of MP, MEB and PP and it was calculated by injecting solution and the values were recorded.

Table 1: Linearity results for MP, MEB AND PP

MP		MEB		PP	
conc. µg/ml	peak area	conc. µg/ml	peak area	conc. µg/ml	peak area
3.6000	2.8010	40.0000	36.6100	0.4000	0.2550
4.0500	3.1910	45.0000	41.8450	0.4500	0.2910
4.5000	3.5730	50.0000	46.6780	0.5000	0.3250
4.9500	3.9330	55.0000	51.6880	0.5500	0.3560
5.4000	4.2670	60.0000	56.0110	0.6000	0.3900

Table 2: System precision for MP, MEB and PP

Statistics	MP		MEB		PP	
	RT	peak area	RT	peak area	RT	peak area
	2.83	3.586	4.14	46.720	4.75	0.325
	2.83	3.577	4.14	46.658	4.75	0.324
	2.83	3.580	4.14	46.642	4.75	0.326
	2.83	3.583	4.14	46.676	4.75	0.326
	2.83	3.578	4.14	46.624	4.75	0.325
	2.83	3.582	4.14	46.693	4.75	0.323
Average	2.83	3.5810	4.14	46.6688	4.750	0.3248
St. Dev.	0.000	0.003	0.000	0.035	0.000	0.001
% RSD	0.000	0.093	0.000	0.075	0.000	0.360

RESULTS AND DISCUSSION

The present investigation reported is a new RP-HPLC method development and validation of simultaneous estimation of MP, MEB and PP. The method developed was proceeding with wavelength selection. The optimized wavelength was 247nm.

In order to get the optimized RP-HPLC method various mobile phases and columns were used. From several trials final method is optimized with the following conditions:

The mobile phase consisted of: Methanol, 0.05M monobasic potassium phosphate, Acetonitrile (48:32:20v/v) and the column used was (Inertsil ODS-3V) C_{18} Column (250mm×4.6mm, 5µm particle size). The flow rate was adjusted to 1.5ml/min. The instrument was operated at 30°C temperature. The purity analysis was performed over a wavelength range of 200-400nm. The injection volume was 15µL. The specificity of the method was to determine whether there are any interference of any impurities (the presence of components may be unexpected to present) in retention time of analytical peak.

The linearity was determined as linearity regression of the claimed analyte concentration of the range 3.6-5.4µg/ml (MP), 40-60µg/ml (MEB), and 0.4-0.6µg/ml (PP). The calibration curve obtained by plotting peak area versus concentration and presented in Table 1 was linear and the squared correlation coefficient was found to be of 0.999, 0.999 and 0.9994 for MP, MEB and PP respectively.

Table 3: Method precision for MP, MEB and PP

Statistics	MP		MEB		PP	
	RT	peak area	RT	peak area	RT	peak area
	2.85	3.577	4.17	46.700	4.78	0.324
	2.85	3.587	4.17	46.661	4.78	0.324
	2.85	3.581	4.17	46.635	4.78	0.325
	2.85	3.582	4.17	46.636	4.78	0.326
	2.85	3.575	4.17	46.670	4.78	0.325
	2.85	3.580	4.17	46.710	4.78	0.324
Average	2.85	3.5803	4.17	46.6687	4.7800	0.3247
St. Dev.	0.000	0.004	0.000	0.031	0.000	0.001
% RSD	0.000	0.117	0.000	0.067	0.000	0.251

Table 4: Ruggedness values for MP, MEB and PP

Statistics	MP		MEB		PP	
	RT	peak area	RT	peak area	RT	peak area
	2.84	3.575	4.15	46.690	4.76	0.325
	2.84	3.577	4.15	46.671	4.76	0.325
	2.84	3.581	4.15	46.655	4.76	0.324
	2.84	3.580	4.15	46.668	4.76	0.326
	2.84	3.576	4.15	46.710	4.76	0.324
	2.84	3.580	4.15	46.710	4.76	0.325
Average	2.84	3.5782	4.15	46.6840	4.7600	0.3248
St. Dev.	0.000	0.002	0.000	0.023	0.000	0.001
% RSD	0.000	0.069	0.000	0.049	0.000	0.232

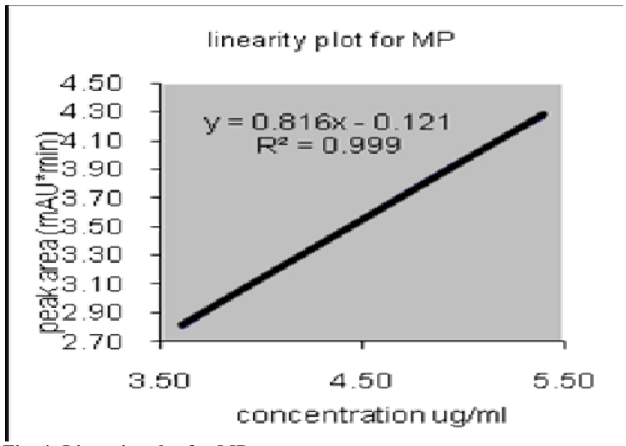

Fig. 4: Linearity plot for MP

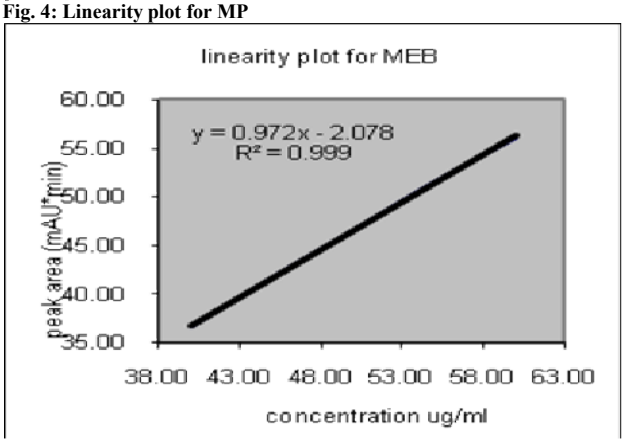

Fig. 5: Linearity plot for MEB

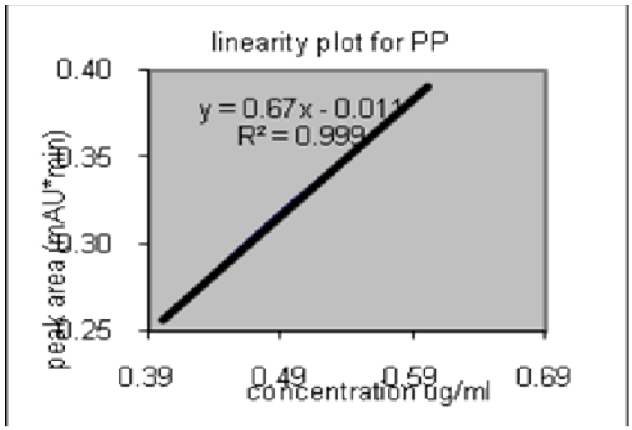

Fig. 6: Linearity plot for PP

Table 5: % Recovery for MP

Concentration at specific level	Active drug added (mg)	Recovered amount (mg)	Mean Recovered
80%	36	36.02	
100%	45	45.15	100.1%
120%	54	54.01	

Table 6: % Recovery for MEB

Concentration at specific level	Active drug added (mg)	Recovered amount (mg)	Mean Recovered
80%	400	400.4	
100%	500	500.8	100.1%
120%	600	600.8	

Table 7: % Recovery for PP

Concentration at specific level	Active drug added (mg)	Recovered amount (mg)	Mean Recovered
80%	4	3.979	
100%	5	4.98	99.3%
120%	6	5.94	

Table 8: System suitability values

MP		MEB		PP	
Theoretical plates	Tailing factor	Theoretical plates	Tailing factor	Theoretical plates	Tailing factor
7864	1.29	7301	1.24	9532	1.23
Resolution between MP and MEB				8.2	
Resolution between MEB and PP				3.2	

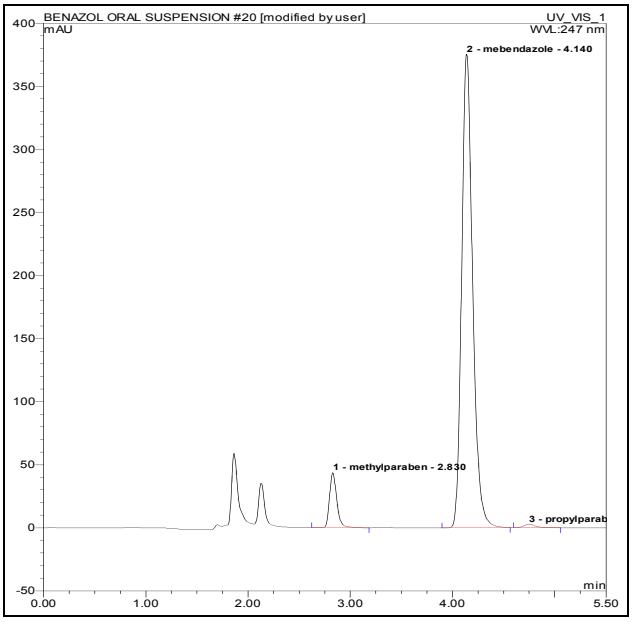

Fig. 7: Chromatogram for Standard Solution

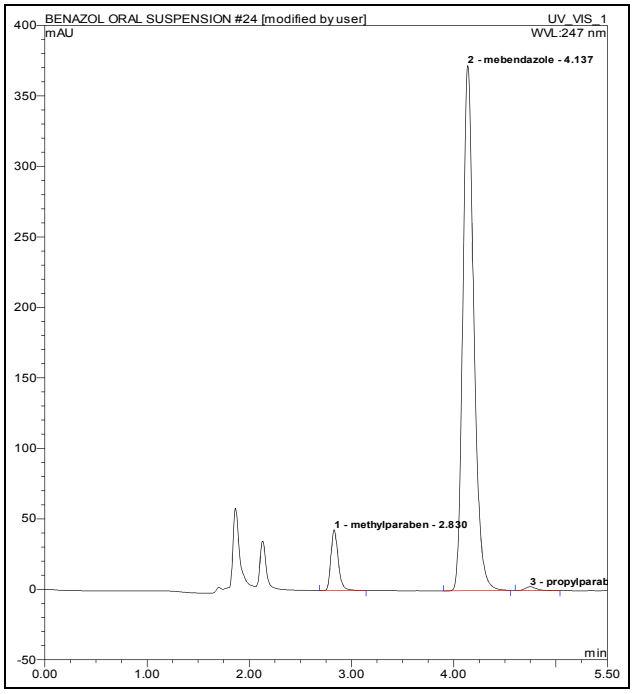

Fig. 8: Chromatogram for Test Solution

The precision of the method was ascertained from determinations of peak areas of six replicates of sample solution. The %Relative Standard Deviation for system precision presented in Table 2 was found to be 0.093, 0.075 and 0.36, the % Relative Standard Deviation for method precision presented in Table 3 was found to be 0.117, 0.067and 0.251and the % Relative Standard Deviation for ruggedness presented in Table 4 was found to be 0.069, 0.049 and 0.232 for MP, MEB and PP respectively. The accuracy study was performed in 80%, 100% and 120% .The percentage recovery was determined for MP, MEB and PP and was found to be 100.1%, 100.1 and 99.3% presented in Tables 5, 6 & 7. The robustness were carried out with minor but deliberate changes in parameters i.e., detection wavelength, column temperature, and flow rate. Theoretical plates and tailing factor were observed and were found to be 7864, 7301 and 9532 (theoretical plates) and 1.29, 1.24 and 1.23(tailing factor) for MP, MEB and PP respectively. The resolution was found to be 8.2 between MP and MEB, and 3.2 between MEB and PP presented in Table8**.** And the Relative Standard Deviation in retention time was found to be zero for MP, zero for MEB and zero for PP.

The method was found to be precise accurate and linear for determination of MP, MEB and PP. The method was developed and validated for system suitability linearity, specificity, accuracy, robustness and ruggedness. All parameters tested were found to be within limits. The study indicates that the method has a significant advantages in term of shorter analysis run time, good resolution between active drugs and the two preservatives MP and PP, high purity of active drug and preservatives peaks, accuracy and precision.

ACKNOWLEDGMENTS

Authors are thankful to Sama Pharmaceuticals Manufacturing Co. for providing facilities to execute the research work.

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