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Development and Validation of Parabens in Cofendyl Syrup

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ABSTRACT

A new simple and rapid high performance liquid chromatography (HPLC) method was developed for the determination of methylparaben and propylparaben in Cofendyl syrup by using cosmosil C18 5 µm, 15cm with 4.6 mm. A mixture of methanol and water of 50:50 with flow rate 1.5 ml/min was used as elutant. The eluents were detected at 254 nm. The retention times of methylparaben and propylparaben were found to be 3.243 min and 10.478 min, respectively. The proposed method was validated with precision, linearity, system suitability, specificity, accuracy and range. The optimum conditions for analysis of the drug were established. The results of analysis were validated by recovery studies. Method showed good reproducibility and recovery, this is evident from %RSD which is less than 2%.The method was found to be simple, accurate, precise and economical.

Keywords: Simultaneous estimation, HPLC, Cofendyl syrup, methylparaben and propylparaben.

INTRODUCTION

Methylparaben[1] which is also defined as methyl 4–paraben or methyl parahydroxybenzoate and propyl paraben[2] is also known as propylparahydroxyl benzoate chemically known as propyl 4-paraben these both drugs having antimicrobial pharmacological activity that is widely used in pharmaceutical preparations for preparation of syrups as a preservatives. Therefore, the present work was aimed to develop and validate [3] a new RP- HPLC method.

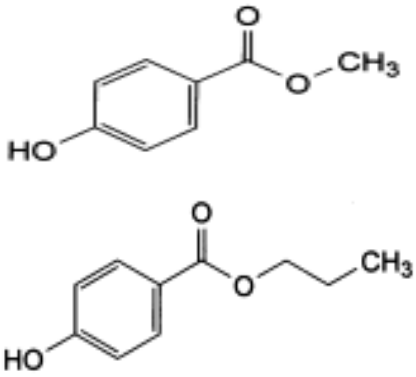

Figure 1: The structures of methylparaben and propylparaben

MATERIALS AND METHODS

Chemicals and Reagents: Cofendyl syrup drug sample was provided by Aspen pharmaceuticals, HPLC grade methanol were obtained from Merck, Germany. Milli-Q water was prepared from Millipore water system.

Instrumentation

An HPLC system (Agilent technologies1200 Series) consisting with online degasser equipped with system controller, Data was acquired and computed by an Empower. The analytic column used to achieve the chromatographic separation was a stainless steel cosmosil C18 5 µm (150 x 4.6 mm) was used for the experiment.

Chromatographic Conditions

The mobile phase was consisted of Methanol and Water 50:50 (v/v). The mobile phase was sonicated for 15 min and filtered through a 0.45 µ membrane filter paper. Flow rate of mobile phase was 1.5 ml/min. The variable wavelength UV–visible detector was set at 254 nm and injection volume was taken as 5 µl. All analyses were performed at ambient temperature. Methylparaben concentration is 0.2mg/ml solution and propylparaben concentration is 0.06mg/ml solution. Mix well and filter through a 0.45 µm filter.

RESULTS AND DISCUSSION

The analytical performance of the method of analysis was checked for specificity, system suitability, accuracy and method precision.

Specificity

Specificity of an analytical procedure is its ability to assess unequivocally the analyte in the presence of components that may be expected to be present.

The solvent and placebo solutions must contain no components, which does not co-elute with the methyl and propylparaben. The peak purity results from the photo diode-array analysis must show that the methylparaben and propylparaben peak is pure i.e. the purity angle (PA) must be less than the threshold angle (TH).

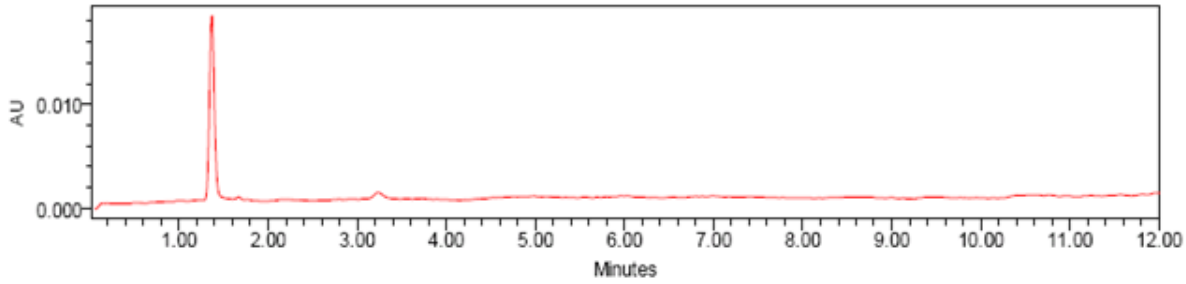

Figure 2: Solvent – No significant peak detected

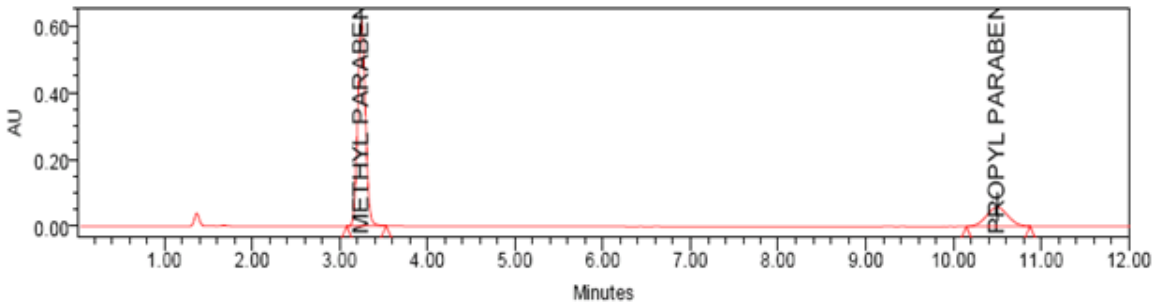

Figure 3: Drug active – Peak due to methylparaben and propylparaben

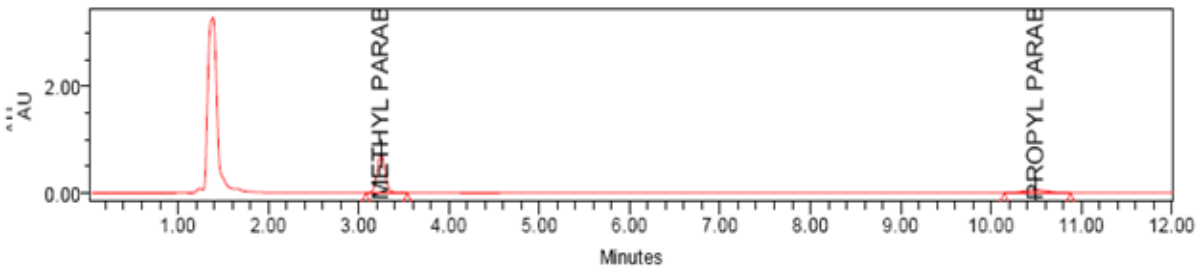

Figure 4: Product – Peak due to methylparaben and propylparaben

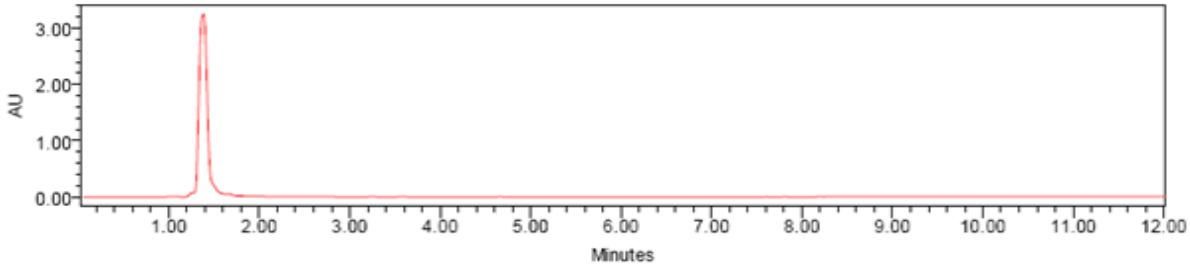

Figure 5: Placebo – no significant peak detected

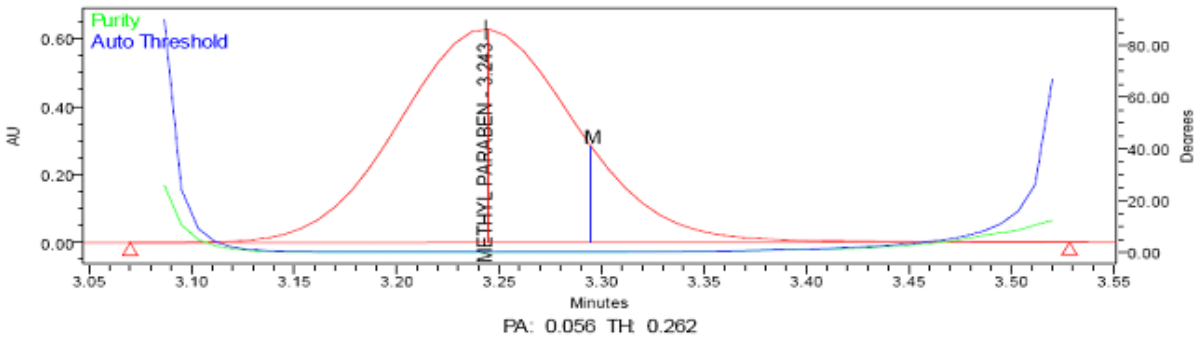

Figure 6: Peak purity of methylparaben

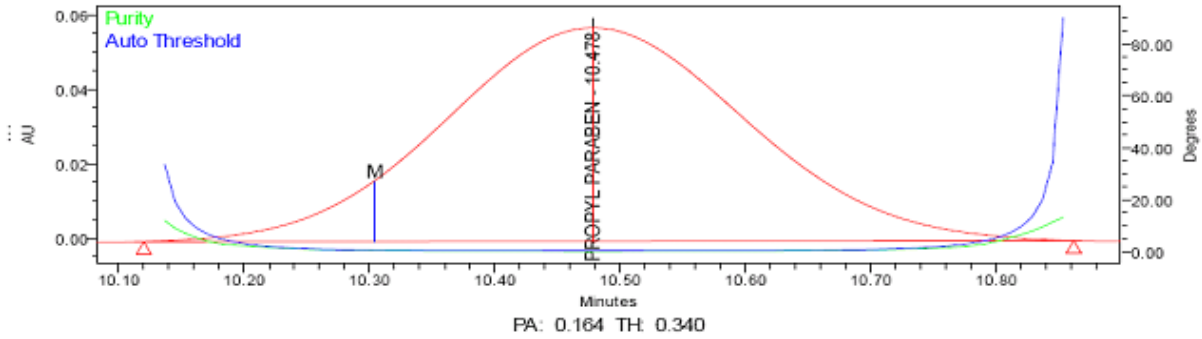

Figure 7: Peak Purity of Propylparaben

Methylparaben and propylparaben are stable under UV light exposure. No components are seen to co-elute with methylparaben and propylparaben peaks, and the peak purity results indicate that methylparaben and propylparaben peak can therefore be considered spectrally pure.

System suitability

System suitability is a measure of the performance and chromatographic quality of the total analytical system i.e. instrument and procedure.

The % RSD of the peak responses due to methylparaben and propylparaben for the six replicate injections must be less than or equal to 2.0 %. Six replicate injections of working standard solution were injected. The percentage relative standard deviation (% RSD) for the peak responses was determined.

Table 1: Injection precision of parabens

Sample	Methylparaben Area (AU)	Propylparaben Area(AU)
Standard	3869895	974210
Standard	3886203	982151
Standard	3902138	994504
Standard	3904891	984379
Standard	3901333	984401
Standard	3903373	987523
Mean	3894639	984528
%RSD	0.4	0.7

The analytical system complies with the requirements specified by the system suitability.

Linearity

The linearity of an assay method is its ability to elicit test results, which are directly proportional to the concentrations of drug actives in samples in a given range. Proof of linearity, justifies the use of single-point calibrations.

The correlation coefficient of the regression line for methylparaben and propylparaben should be greater than or equal to 0.99.The Y-intercept of the line should not be significantly different from zero, i.e. the assessment value (z) falls within the specified limits only when + 5 > z > - 5. Five solutions containing 50, 75, 100, 125, and 150 % of methylparaben and propylparaben, relative to the working concentrations, were prepared and injected according to the method of analysis. A linear regression curve was constructed, and the correlation coefficients (R^2) and assessment values calculated.

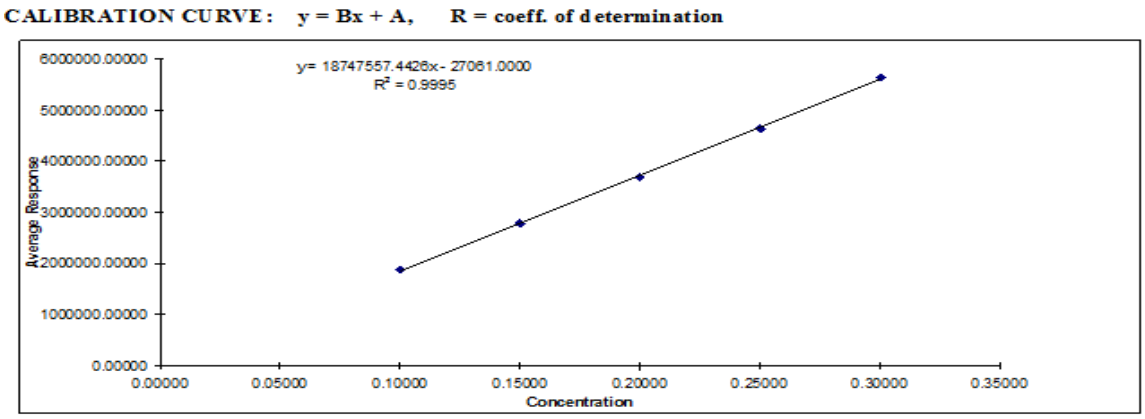

Figure 8: Linearity of methylparaben

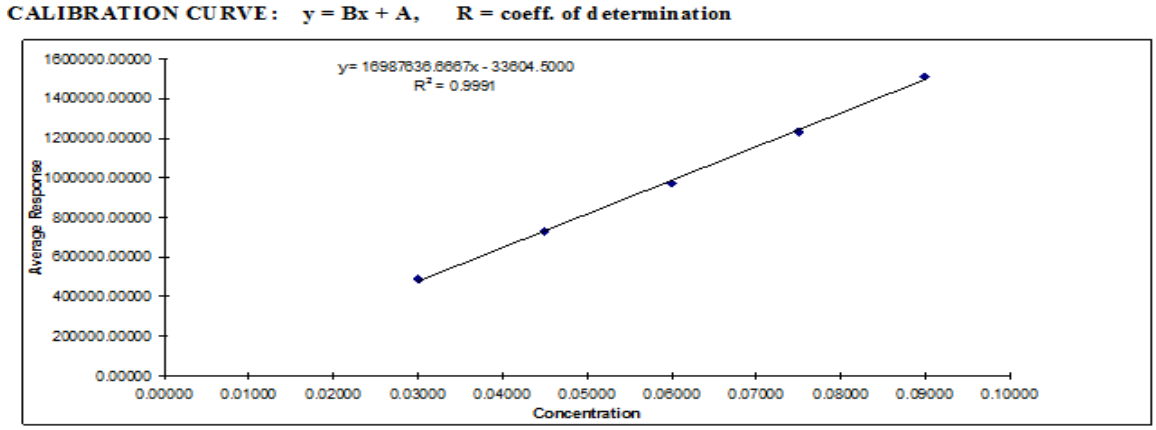

Figure 9: Linearity of propylparaben

Table 2: Regression data of parabens

Linearity Experiment	Methylparaben	Propylparaben
Range (mg/ml)	0.10-0.30	0.030-0.090
Regression Coefficient	0.9995	0.9991
Assessment value(z)	-1	-3
Retention time	3.247	10.478

The correlation coefficient for methylparaben and propylparaben are 1.00 and 1.00 respectively. The graph is a straight line and the assessment values for methylparaben and propylparaben are -1 and -3 respectively.

Accuracy

The accuracy of an analytical method expresses the closeness of test results obtained by that method to the true value.

The percentage recovery of the preservative compounds, for each solution prepared, must be within 95.0 – 105.0 % of the actual amount. Sample solutions were weighed with known concentrations of methylparaben and propylparaben to result in concentrations representing respectively 50, 75, 100,125, and 150 % relative to the working concentrations. The samples were injected in duplicate according to the method of analysis.

Table 3: Accuracy of methylparaben

Sample (%)	Theoretical	Actual	% Recovery	Average % Recovery
50	0.058	0.059	101.7	101.7
		0.059	101.7	
75	0.087	0.089	102.3	101.7
		0.088	101.1	
100	0.116	0.116	100.0	100.0
		0.116	100.0	
125	0.145	0.145	100.0	100.4
		0.146	100.7	
150	0.174	0.170	97.7	97.4
		0.169	97.1	

Table 4: accuracy of propylparaben

Sample (%)	Theoretical	Actual	% Recovery	Average % Recovery
50	0.014	0.014	100.0	100.0
		0.014	100.0	
75	0.021	0.021	100.0	100.0
		0.021	100.0	

100	0.028	0.028	100.0	100.0
		0.028	100.0	
125	0.035	0.036	102.9	102.9
		0.036	102.9	
150	0.042	0.042	100.0	100.0
		0.042	100.0	

From the accuracy results above, the percentage recovery values for methylparaben and propylparaben satisfy the acceptance criteria for accuracy across the range of 50 -150%.

Repeatability

This parameter determines the repeatability of preservative results under the same operating conditions over a short period of time.

The % RSD due to methylparaben and propylparaben for the six samples must be less than or equal to 2.0 %. Six separate sample preparations were analysed according to the method of analysis.

Table 5: Repetability of parabens

Sample	Methylparaben(%m/v)	Propylparaben(%m/v)
Standard	0.118	0.030
Standard	0.118	0.030
Standard	0.118	0.030
Standard	0.118	0.030
Standard	0.118	0.030
Standard	0.118	0.030
Mean	0.118	0.030
% RSD	0.00	0.00

Intermediate Precision

Intermediate Precision of an analytical procedure expresses intra-laboratory variations of the repeatability test performed by a different analyst, on a different day, and using different reagents, mobile phases and solvents.

The % RSD due to methylparaben and propylparaben concentration for the six samples must be less than or equal to 3.0 %. The mean results obtained in the repeatability, and the mean results for the intermediate precision must not differ by more than 4.0 %. Six separate sample preparations were assayed according to the method of analysis.

Table 6: Precision of methyl and propylparabens

Sample	Methylparaben(%m/v)	Propylparaben(%m/v)
Standard	0.118	0.030
Standard	0.115	0.029
Standard	0.114	0.029
Standard	0.114	0.029
Standard	0.115	0.029

Standard	0.115	0.029
Mean	0.115	0.029
% RSD	1.3	1.4
Tests	Mean Results (% m/v)	Mean Results (% m/v)
	Methylparaben	Propylparaben
Repeatability	0.118	0.03
Intermediate Precision	0.115	0.029
Mean	0.117	0.03
% RSD	1.8	2.4

The RSD for the mean results of repeatability and the mean results of the intermediate is less than 2.0%.

Range

Range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Based on the accuracy results, the range for the preservatives of Cofendyl syrup is 0.06 – 0.18 % m/v for methylparaben and 0.015 - 0.045 % m/v for propylparaben. This represents 50 % to 150 % of the working concentration.

APPLICATIONS

The present study is useful especially in pharmaceutical dosage form and drug concentration monitoring. The method also be readily adapted for routine quality control analysis.

CONCLUSION

The RP-HPLC method for the determination of Cofendyl syrup is validated in this study has acceptable correlation coefficient, RSD (%) and deviation which makes it versatile and valuable in many applications.

ACKOWLEDGEMENT

The authors are grateful to Aspen pharmaceuticals limited for their continuous support and help.

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