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Development and validation of RP-HPLC method for simultaneous determination of paraben preservatives in pharmaceutical liquid dosage

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Abstract
A reversed phase HPLC method has been developed that allows the simultaneous estimation of the methyl paraben (MP) and propyl paraben (PP) in pharmaceutical liquid dosage form. The mobile phase was filtered and degassed mixture of buffer (pH 2.0) and acetonitrile (68: 32). The detector wavelength was set at 205 nm and flow rate was 1.0 mL/min. Under these conditions, separation of the two components was achieved in less than 10 min. Validation parameters allow to consider the method adequate. The method was successfully used for estimating both paraben preservatives in pharmaceutical liquid formulation.

Keywords: methyl paraben, propyl paraben, validation, HPLC, estimation

Introduction
Preservatives are antimicrobial agents that are commonly used in pharmaceutical product to protect the health of the patient, as well as to keep the potency and stability of the product during shelf life [1]. For preservatives to be effective, they must reduce microbes and prevent further growth [2]. Among various formulations, Liquid formulations are more susceptible to microorganisms due to nature of their ingredients. Parabens are most commonly used preservatives in liquid pharmaceutical preparations. Now day’s combinations of two or more parabens are used to increase the system's ability to resist microbial contamination and expand the range of micro-organisms against which pharmaceutical products are protected [3]. Methyl paraben (MP) and propyl paraben (PP) are the most commonly used parabens and are often used together since they have synergistic effects. An excess of these preservatives can be harmful to human health. Therefore, the minimum acceptable concentrations of parabens are controlled by regulation, and the quantitative analysis of these preservatives is important for the routine analysis of pharmaceutical products. The analytical determination of these preservatives is important not only for quality assurance, but also for consumer interest and protection [4].
Numerous existing analytical procedures, HPLC and other techniques, are reported in the literature for the quantification of the paraben alone or in combination [5-8]. Such a method is required as there look to be an increasing use of combination of preservatives in pharmaceutical formulations, food industry and cosmetic products. Furthermore, many of the methods described use complicated and laborious pretreatment procedures such as steam distillation, multiple passages and solid phase extractions. Therefore, the aim of this work was to develop and validate simple HPLC method for simultaneous estimation of the methyl and propyl paraben liquid pharmaceutical product.

Material and Methods
Materials
Methyl *p*-hydroxybenzoate (methylparaben) and propyl *p*-hydroxybenzoate (propyl paraben) were received from Sigma-Aldrich. HPLC grade acetonitrile was purchased from SD Fine Chemicals Ltd. and reagent grade citric acid, phosphoric acid, sodium dihydrogen phosphate and ethylenediamine tetraacetate were from Signet Chemical Corporation Sodium hydroxide was from Colorcon Asia Pvt. Ltd and water used was deionized laboratory water.

Instrument
A Waters e2695 model HPLC equipped with quaternary pump for 1-4 solvent flow ranges from 0.000 to 10.000 mL/min, variable wavelength programmable UV/VIS detector (Waters 2489), column compartment with heater 20 to 65°C (in 1°C increment), Empower 2 software was used for the analysis of drug.

Chromatographic conditions

The mobile phase was filtered and degassed mixture of buffer (pH 2.0) and acetonitrile (68: 32).Known volume (e.g., 50 µL) of standard or sample solution was injected. The flow rate was 1.0 mL/min and the UV detector was set at 215 nm.

Preparation of blank solution

The 5 ml of methanol were mixed with 5 ml of 50% formic acid and diluted to 100 ml with methanol.

Preparation of standard stock solution of propyl paraben

Standard stock solution (0.2 mg/ml) of propyl paraben (PP) was prepared by transferring accurately about 20.00 mg of PP working standard in to a 100 mL volumetric flask. About 70 ml of methanol was added to it and sonicated to dissolve. The volume was then made up with more methanol.

Preparation of standard solution

Standard solution (0.1 mg/ml and 0.01 mg/ml of MP and PP) was prepared by transferring accurately about 20 mg of MP working standard to a 200 ml volumetric flask. About 10 ml of 50% formic acid was added to it and sonicated to dissolve. 10 ml of standard stock solution of PP was added to it and volume was then made up with methanol and mixed. The solution was then filtered through 0.45µ nylon filter.

Preparation of sample solution

An accurately weighed 5 gm of suspension was transferred to a 100 ml volumetric flask and 5 mL of 50% formic acid was added to volumetric flask. Then volume was made up with methanol. The solution was then filtered through 0.45µ nylon filter.

Validation of analytical method

The chromatographic conditions were validated by evaluating specificity, linearity, accuracy, method and intermediate precision, robustness, system suitability and solution stability according to ICH Q2 (R1) guidelines [8]. Linearity of method was verified by analysis in triplicates of five points of standard. Recoveries were determined with the same method by comparing the calculated amount and added amount. Repeatability of method was determined by analyzing six injections of the sample and intermediate precision was assessed by analyzing sample solution by different analyst in different instrument on different day. Robustness was determined to assess method's capability to remain unaffected by small but deliberate variations in method parameters.

Result & Discussion

During the optimization of the method, different chromatographic conditions were employed to optimize the method. Higher resolution and batter peak symmetry for methyl paraben and propyl paraben was found with mobile phase buffer (pH 2.0) and acetonitrile (68: 32) at flow rate 1.0 ml/min as compared to other tested mobile phase and flow rate. The standard solution of was subjected to ultraviolet scanning and λmax was found to be at 215 nm. Therefore on the basis of above observation, the final chromatographic conditions were those mentioned above in previous section. Chromatogram of blank, standard and test sample was depicted in fig. 1-3. There was no interference observed in the chromatogram of standard and test sample. The retention time of methyl paraben and propyl paraben was 4.577 min. and 6.907min respectively. Therefore it was concluded that the method is specific.

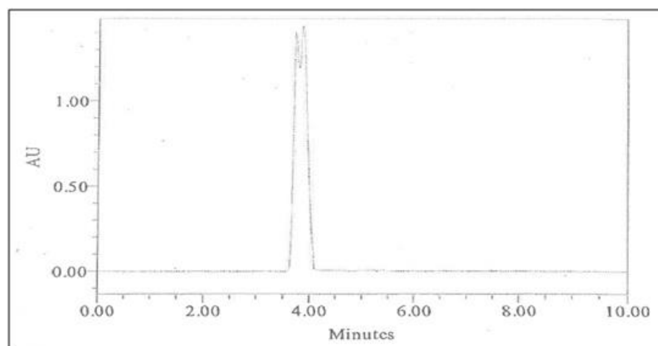

Fig 1: Chromatogram of blank preparation

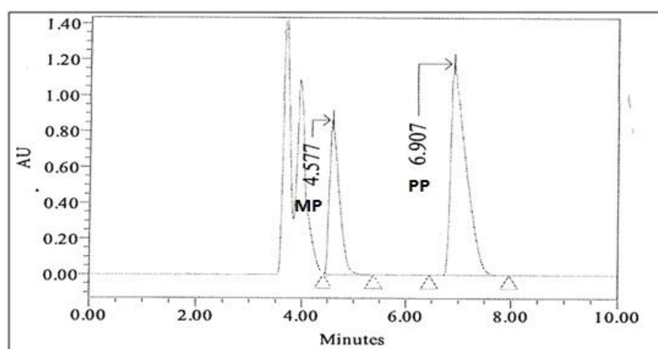

Fig 2: Chromatogram of standard

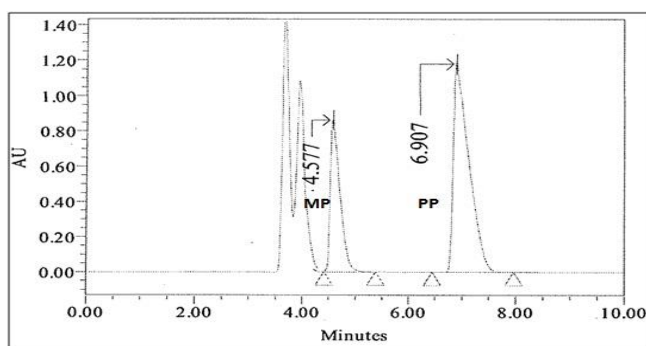

Fig 3: Chromatogram of test sample

The linearity was determined over the range of 160-240 µg/ml of methyl paraben and 16-24 µg/mL of propyl paraben and was graphically presented in fig. 4 and 5 for methyl paraben and propyl paraben respectively. The regression coefficient was found more than 0.999. This indicated that method was linear over specified range for methyl paraben and propyl paraben.

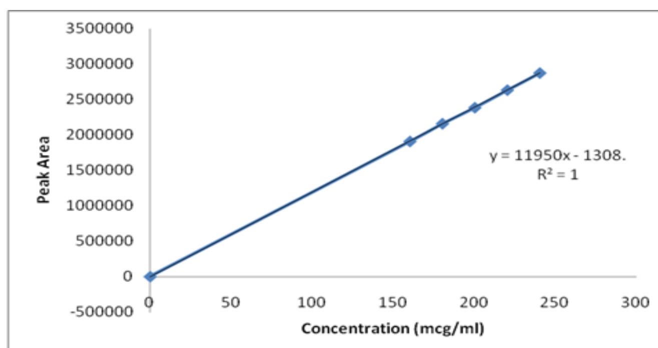

Fig 4: Linearity curve for methyl paraben

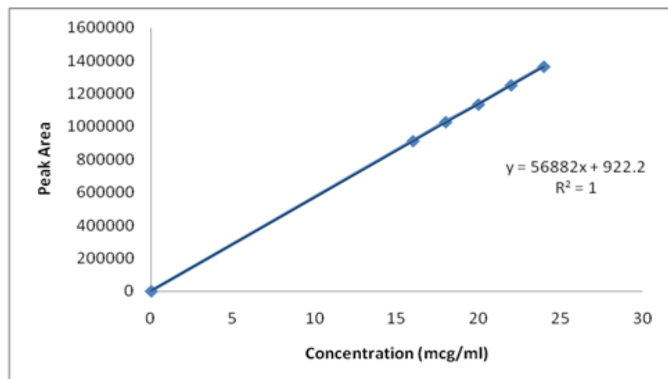

Fig 5: Linearity curve for propyl paraben

Accuracy of the method was calculated by recovery studies at three levels by standard addition method. The mean recoveries for methyl paraben and propyl paraben were found to be 98.62 ± 3.76 and 100.1 ± 3.66 respectively, which are within acceptable limit.

Table 1: Repeatability of method for determination of methyl and propyl paraben

Sample	Methyl Paraben			Propyl Paraben		
	Assay (%)	Mean ± SD	RSD	Assay (%)	Mean ± SD	RSD
Sample 1	98.57	99.42 ± 1.37	1.38	105.39	106.62 ± 1.37	1.79
Sample 2	99.97			108.30		
Sample 3	99.47			105.40		
Sample 4	99.09			109.62		
Sample 5	101.73			106.18		
Sample 6	97.69			104.83		

The method precision (repeatability) was carried out using six different sample preparations from same homogenous blend of marketed sample. The relative standard deviation (R.S.D) for sample preparation is 1.38 and 1.79 respectively of methyl paraben and propyl paraben. The RSD for all sample was found to be less than 2, hence method is precise (table 1).

Table 2: Intermediate precision of method for determination of amino acids

Amino acid	Assay (%)		F test parameter	
	Day I	Day II	F *critical*	F *calculated*
Methyl paraben	99.42 ± 1.38 (1.38)	99.97 ± 1.33 (1.33)	5.05	1.069
Propyl Paraben	106.62 ± 1.91 (1.79)	105.54 ± 1.46 (1.39)	5.05	1.69

Intermediate precision of method was determined by same sample preparation analyzed using different instruments, in two different labs and on different days. The intermediate precision data was shown in table 2. Result showed below 2 % RSD value for both. As evident from table 2 the calculated F value are less than the tabulated F value, indicating the precision of the method with respect to different laboratory, instrument and columns for the analysis of methyl and propyl paraben.

The Robustness was checked by injecting standard and three-sample solutions in single at each different condition with respect to control condition. Robustness of the method was determined by varying the instrumental conditions such as flow rate (±0.2 ml/min), wavelength of detection (±2nm), and change in pH of buffer (±0.2). Sample solution was injected in each condition and assayed for methyl and propyl paraben. The result of the robustness of the assay method is demonstrated in Table 3. The percent assay of both parabens were good under most conditions and did not show a significant change when the critical parameters were modified.

Table 3: Robustness: Effect of alteration in chromatographic conditions

Operating Conditions	Assay (%) ± SD	
	Methyl paraben	Propyl paraben
Set 1	98.93 ± 0.73	103.74 ± 1.51
Set 2	98.23 ± 0.94	102.63 ± 1.24
Set 3	100.73 ± 1.49	100.79 ± 0.81
Set 4	100.59 ± 1.72	99.54 ± 1.32
Set 5	100.12 ± 1.41	99.32 ± 0.71
Set 6	98.4 ± 0.62	97.97 ± 1.41
Set 7	102.75 ± 1.43	98.64 ± 1.09

Set 1: Flow rate (1 ml/min), wavelength (215 nm), pH of buffer (2.0)
Set 2: Flow rate (0.8 ml/min), wavelength (215 nm), pH of buffer (2.0)
Set 3: Flow rate (1.2 ml/min), wavelength (215 nm), pH of buffer (2.0)
Set 4: Flow rate (1 ml/min), wavelength (213 nm), pH of buffer (2.0)
Set 5: Flow rate (1 ml/min), wavelength (217 nm), pH of buffer (2.0)
Set 6: Flow rate (1 ml/min), wavelength (215 nm), pH of buffer (1.8)
Set 7: Flow rate (1 ml/min), wavelength (215 nm), pH of buffer (2.2)

Conclusion

A HPLC method has been developed for simultaneous estimation of methyl and propyl paraben in marketed liquid pharmaceutical formulation. The developed HPLC technique is precise, accurate and robust. Statistical analysis proves that the method is reproducible and selective for the analysis of paraben preservative in pharmaceutical liquid dosage forms.

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