Research Article

Development and Validation of Analytical Method for Determination of Preservatives in Sterile Dosage Form

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ABSTRACT

A simple and robust high performance liquid chromatography (HPLC) method is described for the assay of methyl paraben (MP) and Propyl paraben (PP) in sterile dosage form. The method is selective and stability indicating and all chromatographic conditions were studied to obtain adequate separation of MP and PP from their sterile dosage form. The HPLC separation was carried out on RP C18 analytical column (150mmx4.6mm) used in gradient elution system. The mobile phase flow rate was 0.7ml/min. and the column temperature was ambient. MP and PP were eluted at 3.5, 5.1min., respectively. Detection was carried out at 254 and 210nm and the mobile phase was used Acetonitrile and 0.05% O-Phosphoric acid in ratio 60:40. The method has been validated for accuracy, precision, ruggedness, linearity, LOD and LOQ.

Keywords: Methyl paraben, Propyl paraben, validation, RP-HPLC.

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INTRODUCTION

Pharmaceutical analysis plays a very significant role in quality control of pharmaceuticals through a rigid check on raw material as well as finished product.[1].

Analytical method development and validation play significant role in drug discovery, development, manufacturing of pharmaceuticals and estimation of small molecule.[2]Method validation is the process used to confirm that analytical procedure employed for specific test is suitable for intended use.[3] Analytical method developed using sophisticated instrument such as HPLC, UV, GC, HPTLC have wide application in assuring the quality and quantity of raw material and finished product.[5] The developed analytical method should be accurate, reproducible, robust, precise and commercial, viable one.

Chromatography is a technique used for separation of component of mixture by continuous distribution of component between two phases. High Performance Liquid Chromatographic technique that can separate a mixture of component and used in biochemistry and analytical chemistry identify, quantify and purify the individual component of the mixture.[4]

Preservative

Preservative is a substance that is added to different products such as paints, food, wood, pharmaceutical formulation, biological samples etc. to prevent microbial contamination or to resist any damage to product from chemical reaction.[6] e.g., Methyl paraben, Propyl paraben.

Methyl paraben is a methyl ester of p-hydroxybenzoic corrosive. It is a non-volatile, stable compound utilized as an antimicrobial additive in nourishments, medications and beauty care products for more than 50 years.

Propyl Paraben Methylparaben and propylparaben are the most commonly used parabens and are frequently utilized together since they have synergistic impacts. It had been discovered that the antimicrobial activities of the parabens.[3]

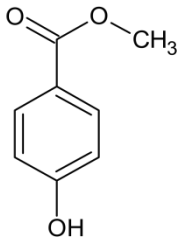

Figure 1: Methyl paraben

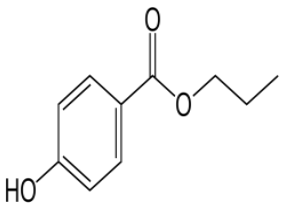

Figure 2: Propyl paraben

Need of determination of preservatives in sterile dosage forms:

Preservatives are commonly used in pharmaceuticals cosmetics, biological samples, food, wood and plastic product to prevent alteration and degradation of the product formulations. However, these preservatives may be harmful to consumer due to their tendency to induce allergic reactions. Hence, the simultaneous determination of these preservatives in commercial pharmaceutical product is particularly important both for quality assurance and consumer safety. Therefore, analytical methodologies developed for the quantification of preservatives in these matrices are usually design to

overcome the problems associated with the interferences which are originated from other constituents.

MATERIALS AND METHODS

Materials and Reagents

Methyl paraben and Propyl parabens were purchased from Alta Lab. Ltd. Mumbai. The used chemicals in all experiment were of analytical grade. HPLC grade acetonitrile ,o-phosphoric acid were used. Fresh double distilled water was used throughout the whole experiment. Genticyn injection IP containing gentamycin-40 mg methyl paraben IP -0.18%w/v and propyl paraben IP -0.02% w/v.

Instrumentation and Chromatographic conditions

The following chromatographic conditions were established by trial and error and were kept constant throughout the experimentation

HPLC Younglin (S.K) GRADIENT System UV Detector

Software : Autochro -3000

Column : 4.6 x 150 mm

Particle size packing : 5 µm

Stationary phase : C_{18} (Cosmosil)

Mobile Phase :- ACETONITRILE ; 0.05 % OPA (60:40)

Detection Wavelength : 210 AND 254 nm

Flow rate : 0.7 ml/min

Temperature : Ambient

Sample size : 20 µl

Prior to use mobile phase were degassed and filtered by passing through a 0.45um pore size by membrane filter.

Preparation of standard stock solution

i. Methyl paraben standard stock solution

An accurately weighed quantity , 10 mg of methyl paraben (MP) was dissolved in methanol in 10 ml volumetric flask and volume was made up to 10.0 ml to produce 1000ug/ml.

ii. Propyl paraben standard stock solution

An accurately weighed quantity, 10 mg of propyl paraben (PP) was dissolved in methanol in 10 ml volumetric flask and volume was made up to 10.0 ml to produce 1000ug/ml.

Preparation of standard solution for chromatogram

Mixed standard solution of MP and PP

i. From freshly prepared standard stock solution (1000ug/ml), aliquots of 0.9ml of MP and 0.1 ml of PP were mixed and diluted appropriately to get final concentration 90ug/ml of MP and 10 ug/ml of PP.

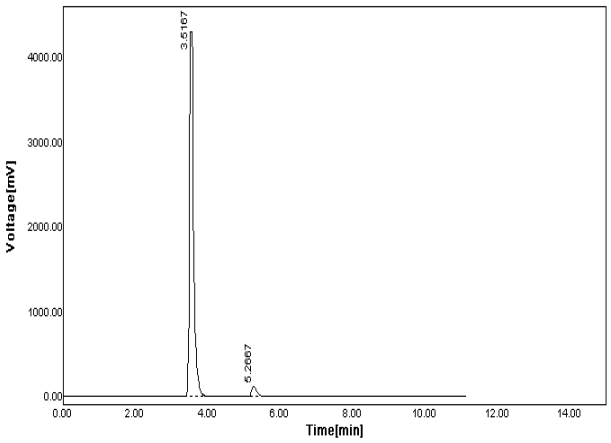

Figure 3: A typical chromatogram of 90ug/ml of MP and 10ug/ml of PP.

Validation study

Accuracy

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

In case of the assay of a drug in a formulated product, accuracy may be determined by application of the analytical method to synthetic mixtures of the drug product components to which known amount of analyte have been added within the range of the method.

Minimum five test concentrations from 80% to 120% are normally used, for establishment of accuracy in assay of drug substance (or finished product). Average recovery should be 99 to 101% of drug at each level.

The accuracy is acceptable if the difference between the true value and mean measured value does not exceed the RSD values obtained for repeatability of the method.

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogenous sample. The precision of an analytical method can be expressed as the standard deviation or relative standard deviation (Coefficient of variation) of a series of measurements.

Precision may be the measure of either the degree of repeatability or reproducibility of an analytical method under normal operating conditions. Reproducibility refers to the use of analytical procedures in different laboratories. Repeatability refers to the use of analytical procedures within a laboratory over short period of time using the same analyst with same equipment. The precision of an analytical method is determined by assaying a sufficient number of aliquots of a homogenous sample to be able to calculate spastically valid estimates of standard deviation or relative standard deviation. In the precision results of all samples should not have RSD ≤ 2%. Repeatability RSD < 2%, Intermediate precision RSD <2%.

Linearity and range

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation proportional to the concentration of analyte in samples within a given range. It should be established across the range of analytical procedure. Linearity is generally reported as the correlation coefficient, the slope of regression line, etc. r ≤ 0.9999.

The range of analytical method is the interval between the upper and lower level of analyte (including these levels) that have been demonstrated to be determined with suitable level of precision, accuracy, and linearity using method written. The range is normally expressed in the same unit as test results (e.g., percentage, parts per billion).

Limit of detection (LOD)

It is the lowest amount of analyte in the sample that can be detected, but not necessarily quantitated under the stated experimental conditions. Thus, limit test rarely substantiates that the amount of analyte is above or below a certain level. It is usually expressed as the concentration of analyte (e.g. percentage, parts per billion) in the sample. Based on the standard deviation of the response (σ) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula

$$LOD = 3.3(\sigma / S) \quad \text{---------- (01)}$$

Limit of quantitation (LOQ)

The limit of quantitation is the lowest amount of analyte in the sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. It is usually expressed as the concentration of analyte (e.g. percentage, parts per billion) in the sample. LOQ may also be calculated based on the standard deviation of the response (σ) and the slope of the calibration curve (S) at levels approximating, the LOQ can be determined according to the formula

$$LOQ = 10(\sigma / S): \quad \text{---------- (02)}$$

RESULT AND DISCUSSION

Analysis of standard drug was done by following parameter:

Melting point

Melting point of drugs were found to be

Methyl paraben: 126-128^{0}C

Propyl paraben: 96-99^{0}C

Solubility:

Methyl paraben : Freely soluble in ethanol (95%), in methanol and very slightly soluble in water.

Propyl paraben: Freely soluble in ethanol (95%), ether, acetone, methanol and very slightly soluble in water.

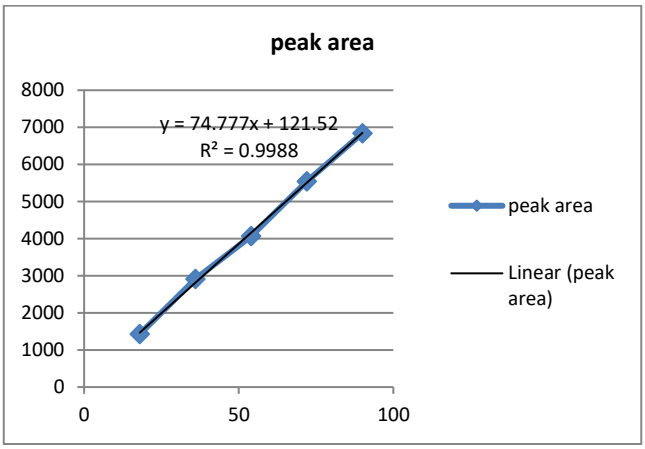

Figure 4: The plot of Linearity and range study for Methyl paraben

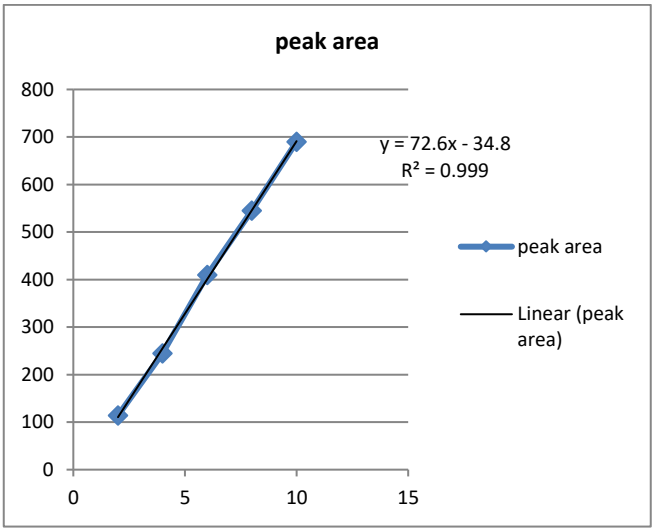

Figure 5: The plot of Linearity and range study for Propyl paraben

HPLC Chromatogram

From the various mobile phases tried, mobile phase containing Acetonitrile and 0.05%orthro- phosphoric acid was selected since it gave sharp, well resolved peaks with summary within the limits and significant reproducible retention time for MP, PP. Chromatogram of MP and PP are shown in fig 6.

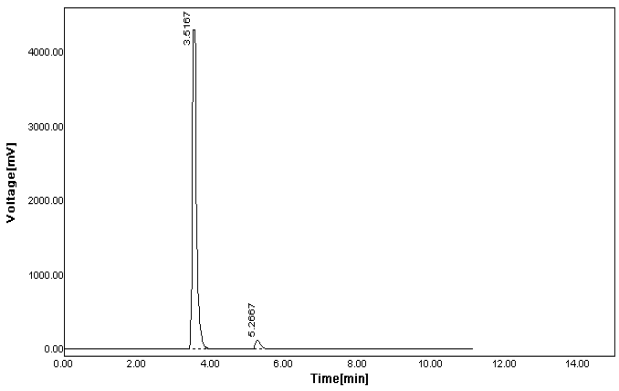

Figure 6: HPLC Chromatogram of MP standard with retention time 3.5 min. and PP of 5.2min.

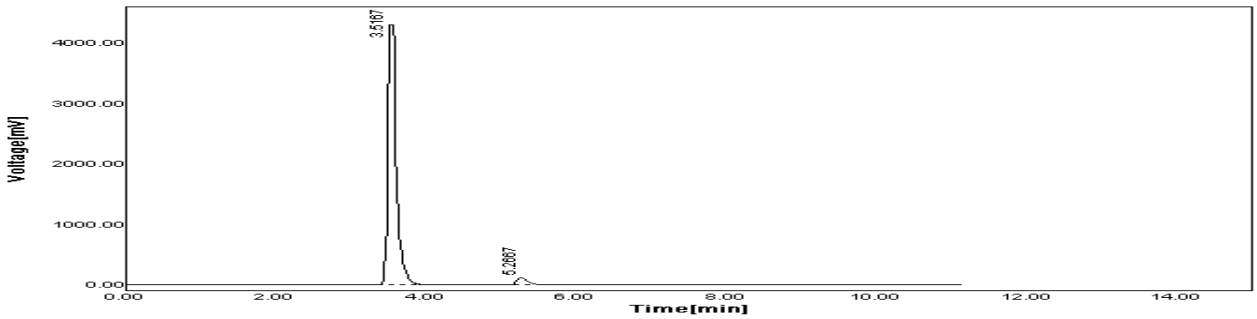

Figure 7: Chromatogram of sterile Marketed preparation of MP, PP showing retention time MP 3.5 and PP 5.2 min.

Accuracy

Table 1: Result and statistical data for recovery study of mixture of MP and PP

Sr. No.	Conc.(ug/ml)		Peak area of std.(mv)		Amount of pure drug added (ug/ml)		Peak area of sample (mv)		%Recovery	
	MP	PP	MP	PP	MP	PP	MP	PP	MP	PP
1	90	10	6840.2853	690.80	72	8	12390.85	1290.85	101.45	98.92
2					90	10	13890.35	1428.35	101.11	98.11
3					108	12	14970.28	1590.68	99.01	98.94
								Mean	100.52	98.656
								±SD	0.9852	0.3866
								%RSD	0.9801	0.3981

Precision

Table 2: Result of precision for mixture of MP and PP

Sr. No.	Conc.(ug/ml)		Peak area of std.(mv)		Peak area of sample(mv)		% Label claim	
	MP	PP	MP	PP	MP	PP	MP	PP
1	90	10	6841.58	689.80	6840.72	691.82	101.20	98.98
2					6841.29	690.58	101.21	98.80
3					6840.48	690.89	101.19	98.95
4					6840.90	691.54	101.20	98.94
5					6840.58	691.92	101.20	98.99
						Mean	101.20	98.91
						±SD	0.0063	0.0746
						%RSD	0.0062	0.0754

Ruggedness

Intraday

Table 3: Result of Intraday for mixture of MP and PP

Sr. No.	Conc.(ug/ml)		Peak area of std.(mv)		Peak area of sample(mv)		% Label claim	
	MP	PP	MP	PP	MP	PP	MP	PP
1	90	10	6840.5853	690.32	6840.7213	691.54	101.20	98.94
2					6841.9850	691.92	101.22	98.99
3					6841.2580	690.82	101.21	98.98
						Mean	101.21	98.98
						±SD	0.008165	0.0216
						%RSD	0.00806	0.2180

Interday

Table 3.1: Result of Interday for mixture of MP and PP

Sr. No.	Conc.(ug/ml)		Peak area of std.(mv)		Peak area of sample(mv)		% Label claim	
	MP	PP	MP	PP	MP	PP	MP	PP
1	90	10	6840.7853	691.80	6840.9852	690.80	101.20	98.92
2					6842.1823	693.52	101.22	99.20
3					6843.5461	695.72	101.24	99.49
						Mean	101.22	99.22
						±SD	0.01613	0.2083
						%RSD	0.01613	0.2104

Different analyst

Table 3.2: Result of Different analyst for mixture of MP and PP

Sr. No.	Conc.(ug/ml)		Peak area of std.(mv)		Peak area of sample(mv)		% Label claim	
	MP	PP	MP	PP	MP	PP	MP	PP
1	90	10	6840.2853	690.80	6842.1240	692.94	101.22	99.12
2					6842.9820	693.85	101.23	99.24
3					6842.7243	693.58	101.23	99.21
						Mean	101.23	99.19
						±SD	0.0047	0.0509
						%RSD	0.0046	0.0514

LOD and LOQ

Table 4: Result of LOD and LOQ

Drug	LOD(ug/ml)	LOQ(ug/ml)
MP	0.016	0.049
PP	0.047	0.142

CONCLUSION

The result of the present study conclusively demonstrated that a simple, selective, precise HPLC method can be developed for the analysis of preservatives in sterile dosage forms. Statistical analysis proves that the method is repeatable and selective for the analysis of methyl paraben and propyl paraben which act as a preservatives in sterile formulations. The proposed method has advantage of convenience for separation of methyl paraben and propyl paraben. The method is also fast as it require approximately 6 min. for analysis. Thus, the proposed HPLC method can be used for quality control of methyl paraben and propyl paraben. The validation parameters were also found acceptable of FDA and ICH guidelines.

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