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NEW VALIDATED HPLC METHOD FOR THE ESTIMATION OF METHYL HYDROXY BENZOATE IN TABLET FORMULATION

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ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Methyl Hydroxy Benzoate in Bulk and Pharmaceutical tablet Formulation. Isocratic elution at a flow rate of 1.0 ml/min was employed on symmetry Luna C18 5µm (4.6 x 150 mm) or equivalent at ambient temperature. The mobile phase consisted of Methanol: Water in the ratio of 50:50 v/v. The UV detection wavelength was 254nm and 20µl sample was injected. The retention time for Methyl Hydroxy Benzoate is identified that 4.8 min. The Average percentage recovery of the method was in the range of 96.1 – 102.6%.The method was validated as per the ICH guidelines. The method was successfully applied for routine quality control analysis of pharmaceutical formulation.

Key Words: Methyl Hydroxy Benzoate, HPLC, UV detection, Recovery, Precise.

INTRODUCTION

Methyl paraben, is a preservative with the chemical formula $CH_3(C_6H_4(OH)COO)$. It is the methyl ester of *p*-hydroxybenzoic acid. Methylparaben serves as a pheromone for a variety of insects[1]. It slows the growth rate in the larval and pupal stages at lower concentrations.[2] Methylparaben and propylparaben are considered generally recognized as safe for food and cosmetic antibacterial preservation.[3] Methylparaben is readily absorbed from the gastrointestinal tract or through the skin.[4] It is hydrolyzed to *p*-hydroxybenzoic acid and rapidly excreted in urine without accumulating in the body.[4] Acute toxicity studies have shown that methylparaben is practically non-toxic by both oral and parenteral administration in animals.[4] and allergic reactions to ingested parabens have been reported.[4]

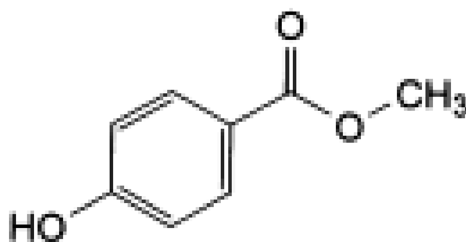


Fig. 1 Structure of Methyl Hydroxy Benzoate

Studies indicate that methylparaben applied on the skin may react with UVB, leading to increased skin aging and DNA damage.[5][6] Ghulam A. Shabir et.al.,[7] analyzed a new and simple Rp-HPLC method has been developed and validated for the simultaneous determination of methyl-4-hydroxybenzoate, propyl-4-hydroxybenzoate and phenothrin in human head lice medicine liquid formulation. The separation was achieved on a Lichrosorb C18, 150 mm × 4.6 mm and 5 μm column with detection wavelength of 254 nm using an isocratic mobile phase mixture of methanol-water (80:20, v/v) at flow rate of 1.0 mL/min. A good linearity correlation was obtained over the investigated concentration ranges. Recoveries of this method for the three components were from 99.50 to 101.00%. The intra-day and inter-day percent relative standard deviations were less than 2.0%. The proposed method was successfully employed for medicine liquid products. Ghulam A. Shabir et.al.,[8] described the use of a reversed-phase C18 column (250 mm×4.0 mm and 5 μm) using a gradient elution system enabled six compounds to be separated simultaneously in a single chromatographic run in less than 12 minutes. The method was successfully validated following ICH guidelines, and it has been demonstrated to be reliable for the assay of benzyl alcohol, ethylene glycol monophenyl ether, methyl hydroxybenzoate, ethyl hydroxybenzoate, propyl hydroxybenzoate, and butyl hydroxybenzoate preservatives used in the manufacturing of drug products. Zhang C et.al.,[9] proposed a method for the contents of adapalene and the preservatives (2-phenoxyethanol and methyl-4-hydroxybenzoate) in adapalene gels. The chromatographic analysis was carried out on a Tigerkin C18 column (150 mm x 4.6 mm, 5 microm). The mobile phase was 0.02 mol/L ammonium acetate buffer (pH 3.0) and tetrahydrofuran-acetonitrile with gradient elution, and the detection wavelength was set at 270 nm. The calibration curves were linear over the ranges of 10-100 mg/L (r = 0.9999), 4-40 mg/L (r = 0.9999) and 4-40 mg/L (r = 0. 9999) for 2-phenoxyethanol, methyl-4-hydroxybenzoate and adapalene, respectively. The average recoveries of the three substances were within 98.0%-98.6%. The method is simple, reliable and suitable for the adapalene gels. G. A. Shabir et.al.,[10] determined for 2-phenoxyethanol, methylparaben, ethylparaben and propylparaben preservatives. The mobile phase consisted of a mixture of acetonitrile, tetrahydrofuran and water (21:13:66, v/v/v), pumped at a flow rate of 1 ml/min. The UV detection was set at 258 nm. A. Chmielewska et.al.,[11] developed a simultaneous determination of fluocinolone acetonide and additives in gel. Drugs were chromatographed on a C18reversed-phase columnwith 55:45 (v/v) methanol–water as mobile phase and detection at 238 nm. Because of the simplicity and accuracy of the method, it was used for routine analysis of fluocinolone cetonide in ointment. Ishiwata H et.al.,[12] determined that Methyl p-hydroxybenzoate (methyl paraben) in foods produced by the Honey Bee was determined by HPLC and confirmed by GC-MS. The compound was detected at a mean concentration of 22.3 +/- 6.8 mg/kg (between 14.2 and 31.9 mg/kg) in commercial royal jelly, but was not detected in honey, propolis or pollen lumps at the detection limit of 1 mg/kg. Fresh royal jelly collected from apiaries contained methyl p-

hydroxybenzoate at a concentration of 20.3 +/- 4.7 mg/kg (between 12.5 and 31.7 mg/kg). These results indicate that the methyl p-hydroxybenzoate in royal jelly is not added but is a natural component.

MATERIALS AND METHODS

Instrumentation

Peak HPLC containing LC 20AT pump and variable wavelength programmable UV-Visible detector and Rheodyne injector was employed for investigation. The chromatographic analysis was performed on a Luna C18 5µm (4.6 x 150 mm) or equivalent. Degassing of the mobile phase was done using a Loba ultrasonic bath sonicator. A Denwar Analytical balance was used for weighing the materials.

Chemicals and Solvents

The reference sample of Methyl Hydroxybenzoate (API) was obtained from Cipla, Mumbai. The Formulation Denorex Dry Scalp Shampoo (Methyl Hydroxybenzoate) was procured from the local market. Methanol used was of HPLC grade and purchased from Merck Specialties Private Limited, Mumbai, India. A mixture of Methanol: Water in the ratio of 50:50 v/v was prepared and used as mobile phase.

Preparation of Standard solution

Accurately weigh 114 mg of Methyl Hydroxybenzoate reference standard into a 100 ml volumetric flask. Dilute and make up to volume with solvent and mix well. Dilute 5 ml of this solution to 100 ml and dilute to volume with solvent. Filter sample through a 0.45 µm filter.

Preparation of Sample Solution

Placed swab in 10 ml of solvent (volume accurately determined). Sonicate for 5 minutes. Squeeze swab out well. Filter sample through a 0.45 µm filter.

Preparation of Blank solution

Placed swab in 10 ml of solvent (volume accurately determined). Sonicate for 5 minutes. Squeeze and swab out well. Filter sample through a 0.45 µm filter. Inject the blank, standard and sample preparation.

Method Development

For developing the method, a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wave length and choosing stationary and mobile phases. The following studies were conducted for this purpose.

Detection wavelength

The spectrum of 10ppm solution of the Methyl Hydroxybenzoate was recorded separately on UV spectrophotometer. The peak of maximum absorbance wavelength was observed. The spectra of Methyl Hydroxybenzoate were showed maximum absorbance at 254nm.

Choice of stationary phase and Mobile Phase

Finally the expected separation and peak shapes were obtained on Luna C18 5µm (4.6 x 150 mm) column. A mixture of Methanol: Water in the ratio of 50:50 v/v was proved to be the most suitable

of all the combinations since the chromatographic peak obtained was better defined and resolved and almost free from tailing.

Flow rate

Flow rates of the mobile phase were changed from 0.5 – 1.5 mL/min for optimum separation. It was found from the experiments that 1.0 mL/min flow rate was ideal for the successful elution of the analyte.

Optimized chromatographic conditions

Chromatographic conditions as optimized above were shown in Table: 1 these optimized conditions were followed for the determination of Methyl Hydroxybenzoate in bulk samples and in its Formulations. The chromatograms for solvent, Drug active, Product, Active – UV Stress, Product – Uv Stress, Placebo and for the Soap are identified. Among all these for the solvent, Placebo and for the soap no significant peaks are detected.

Table 1: Optimized chromatographic conditions for estimation Methyl Hydroxybenzoate

Mobile phase	**Methanol : water 50:50/v/v**
Pump mode	Isocratic
Detector	Uv/Vis
Diluent	Mobile phase
Column	Luna C18 5µm (4.6 x 150 mm)
Column Temp	Ambient
Wavelength	254 nm
Injection Volume	20 µl
Flow rate	1.0 mL/min
Retention Time	4.8 min

Validation Procedure and requirements

The analytical performance of the method of analysis was checked for specificity, System suitability, detection limit, and method precision.

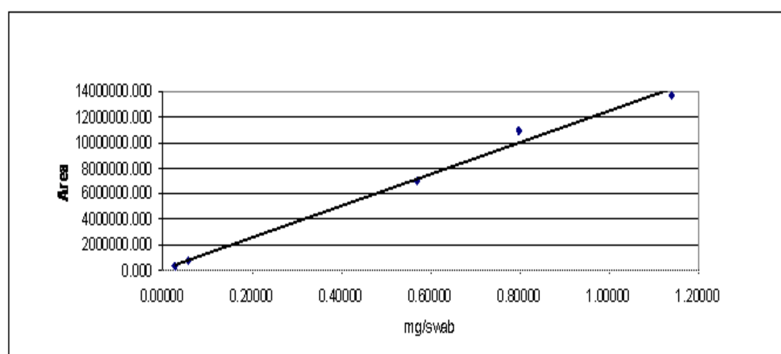
Linearity

From 1.14 mg/ml stock solution a series of standard solutions were prepared. Those solutions are 0.798854mg/swab, 0.57061 mg/swab, 0.057061 mg/swab, 0.0285305 mg/swab and 0.00285305 mg/swab. The range of standard solutions are injected twice and the average result was used in treatment of results. Six solutions containing 1.14122, 0.798854, 0.57061, 0.057061, 0.0285305 and 0.00285305 mg/swab of Methyl Hydroxybenzoate, relative to the working concentrations, were prepared and injected according to the method of analysis. A linear regression curve was constructed, 50% MAC is equal to 0.57 mg/swab and the method gives linear response from 0.00285305- 1.14 mg/swab therefore the method can detect the above concentration of API 0.57061 mg/swab (50% MAC) required by the method.

Table. 2 Linearity results for Methyl Hydroxy benzoate

Conc. (mg/swab)	Area 1	Area 2	Average
1.14	13694020	13652269	13673145
0.798854	10909352	10895749	10902551
0.57061	6913297	692419	3802858
0.057061	699693	703400	701547
0.0285305	358946	355322	357134
0.00285305	27681	29132	284067

Fig.2 Linearity curve for Methyl Hydroxy benzoate



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 co-elute with the Methyl Hydroxybenzoate. The peak purity results from the photo diode-array analysis must show that the Methyl Hydroxybenzoate peak is pure i.e. the purity angle (PA) must be less than the threshold angle (TH). The solutions like solvent, Placebo at working concentration,API at working concentration , Product at working concentration and Detergent solution were injected using the conditions specified in the method of analysis. No components are seen to co-elute with the Methyl Hydroxybenzoate peak, and the peak Purity results indicate that Methyl Hydroxybenzoate peak can therefore be considered spectrally pure. The method employed is specific for the API Methyl Hydroxybenzoate in the product. The Chromatogram results are shown from the Fig: 3 to Fig: 7 and the Peak purity results are shown from Fig: 8 to Fig: 11.

Fig.3 Drug active – Peak due to Methyl Paraben

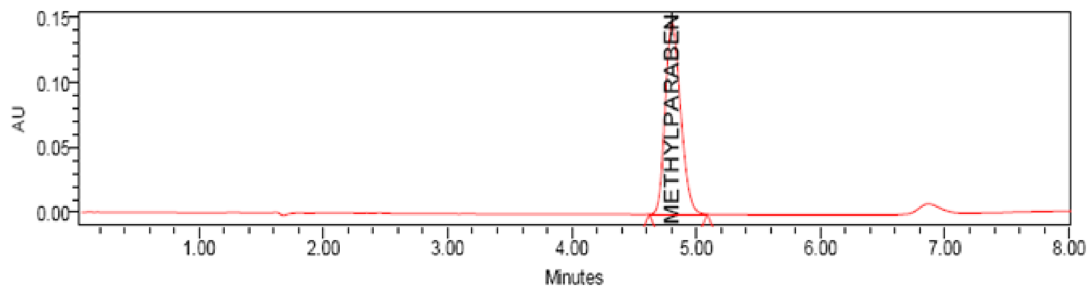


Fig.4 Product – Peak due to Methyl Paraben

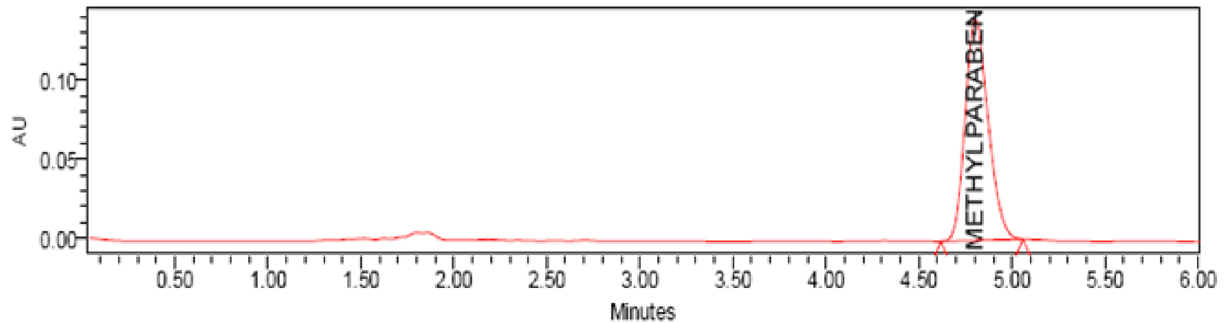


Fig.5 Active – UV stress: Peak due to Methyl Paraben

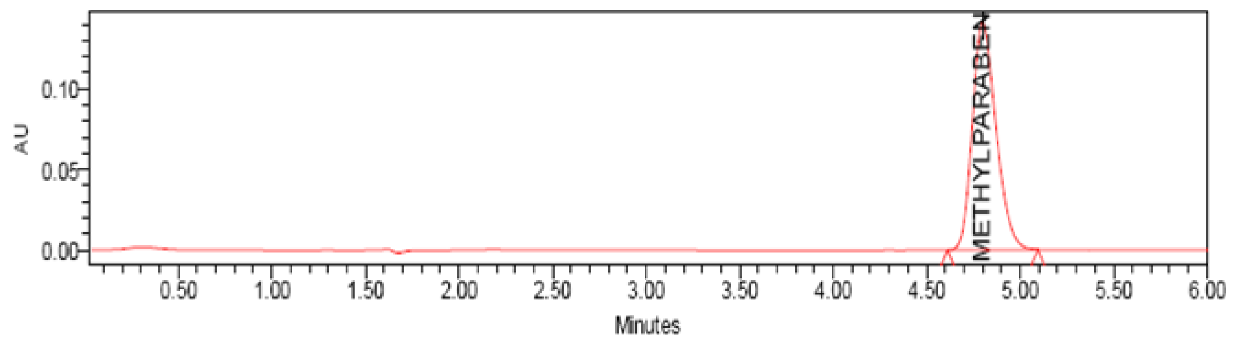


Fig.6 Product – UV stress: Peak due to Methyl Paraben

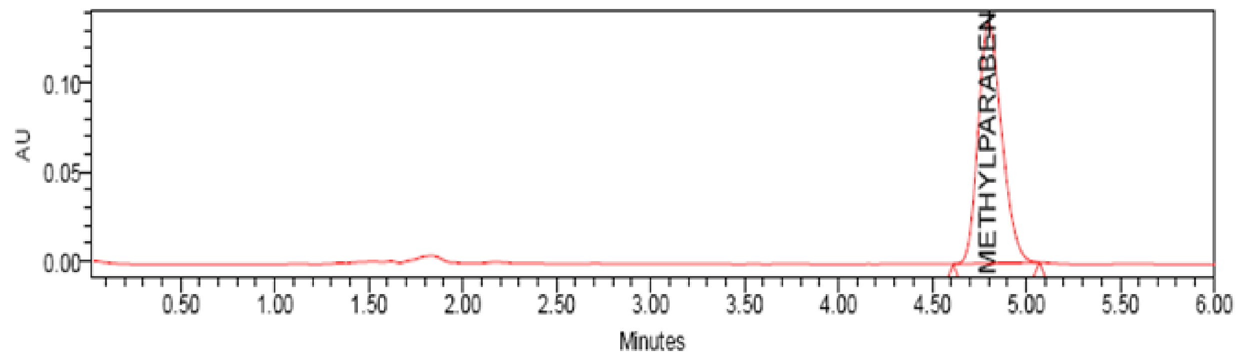


Fig.7 Detergent: No significant peak detected

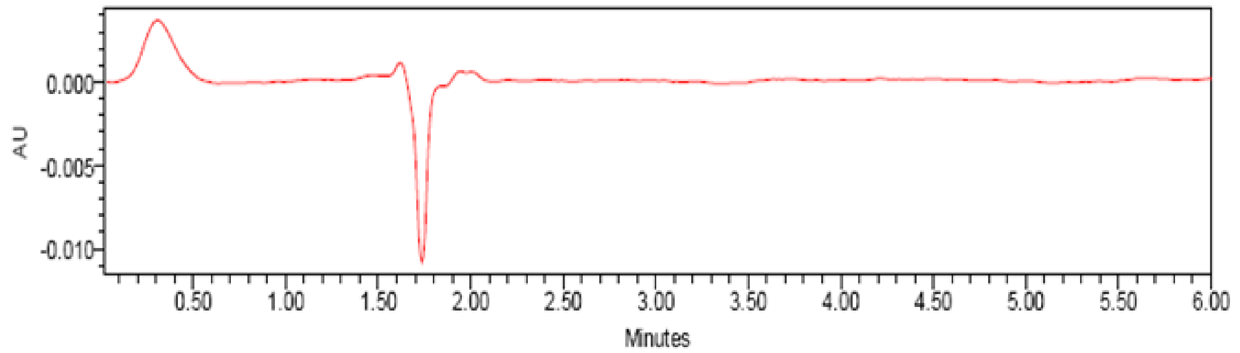


Fig.8: API 0.151 < 0.400(Purity Angle<Threshold Angle)

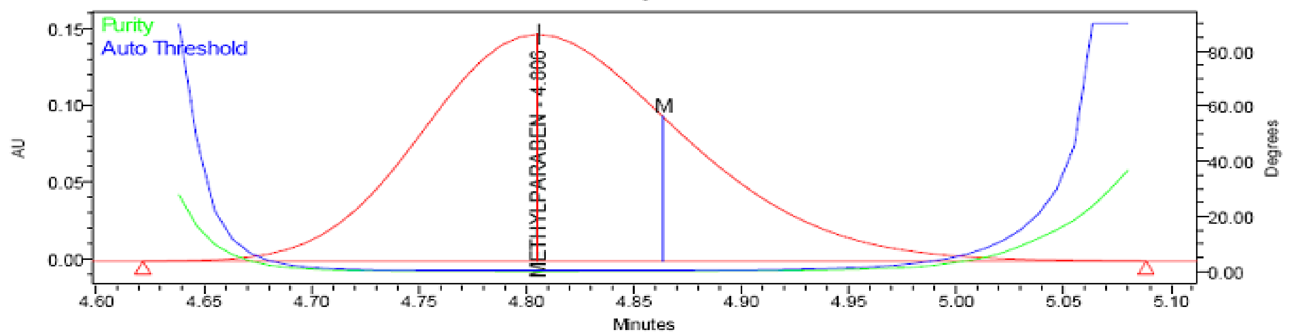


Fig.9: Product 0.135 < 0.303(Purity Angle<Threshold Angle)

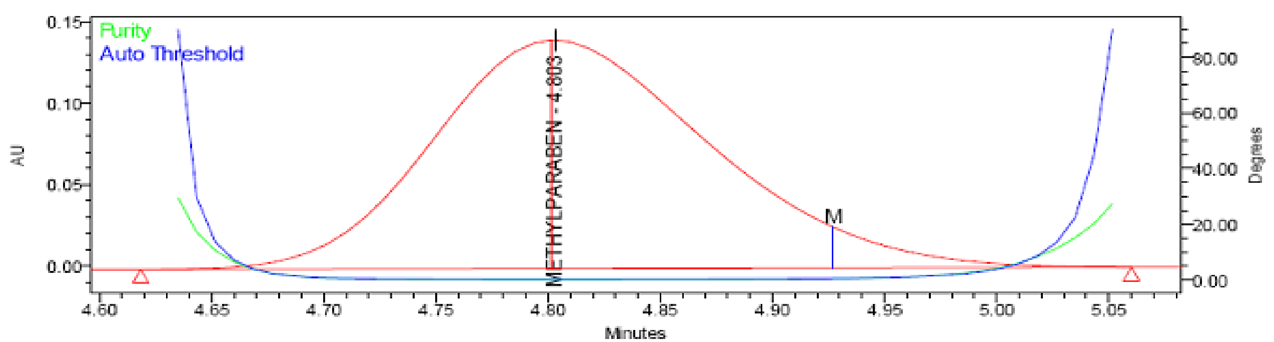


Fig.10: API UV-stressed 0.506 < 0.828 (Purity Angle<Threshold Angle)

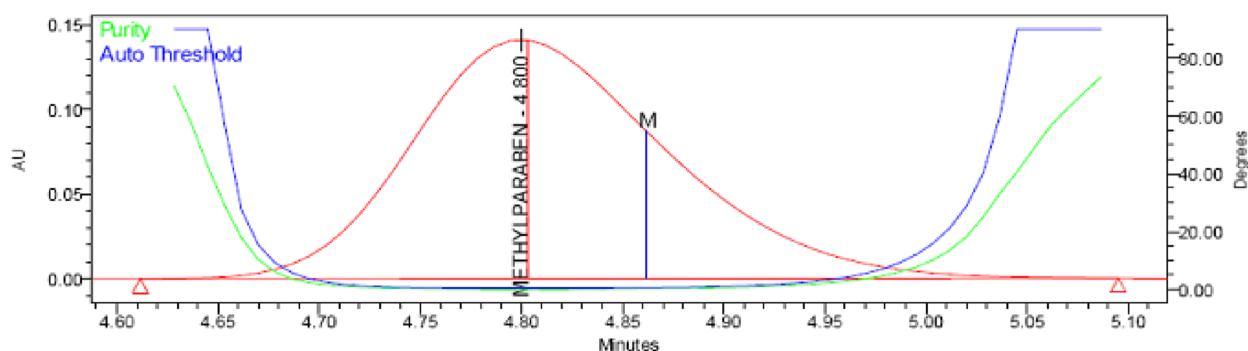
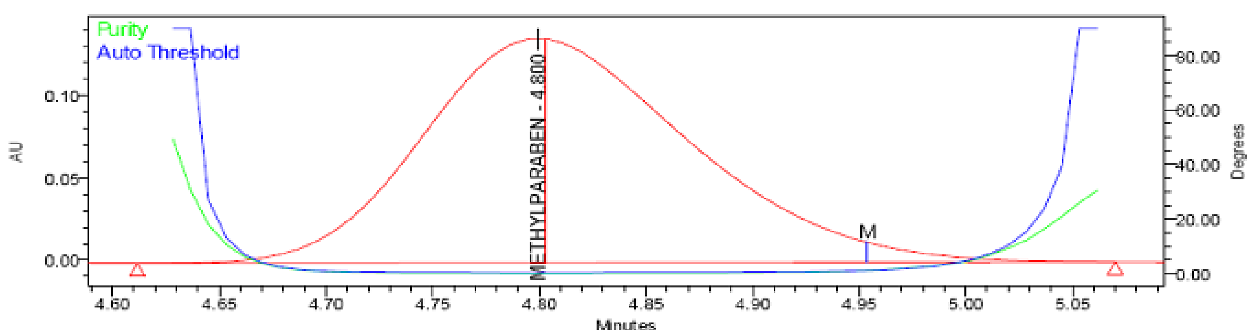


Fig.11: Product UV-stressed 0.170 < 0.348(Purity Angle<Threshold Angle)



System Suitability

System suitability is a measure of the performance and chromatographic quality of the total analytical system – i.e. instrument and procedure. Six replicate injections of API working standard solution were injected according to the method of analysis. The percentage relative standard deviations (% RSD) for the peak responses were determined. The % RSD of the peak responses due to the Methyl Hydroxybenzoate for six injections must be less than or equal to 5.0 %. The analytical system complies with the requirements specified by the system suitability.

Table 3: Results of System Suitability

Sample	Methyl Hydroxybenzoate Area
1	6852001
2	6854085
3	6852371
4	6855567
5	6863937
6	6865373
Mean	6857222
% RSD	0.1

Detection Limit

The limit of detection by definition is a parameter of a limit test. It is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantitated under the stated experimental conditions. It merely substantiates that analyte concentration is above or below a certain level. The Detection Limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be

reliable detected. The maximum allowable carryover of Methyl Hydroxybenzoate is 1.14 mg/swab as determined in the Cleaning Validation Matrix.

Method Precision

The percentage of a test procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. The precision % recovery of a known amount of API in the sample after swabbing.

Swab sampling

An amount of material (predetermined limit) is placed on a specific surface area (stainless steel) and swabbed as outlined in the Cleaning Validation SOP using the specified solvent and specified material. The precision of the analytical method is determined by assaying the swabs and calculating the % Recovery of the API results. The precision will entail repeated testing of six samples prepared in the following manner. Six replicate injections of API MAC working standard solutions were injected according to the method of analysis. The percentages Recovery for the peak responses were determined.

Preparation of Standard and Sample solution

Accurately weigh 114 mg of Methyl Hydroxybenzoate reference standard into a 50 ml Volumetric flask. Add 30 ml of solvent and sonicate for 15 minutes, cool and make up to volume with solvent. Dilute 10 µl of this solution to 10 ml with solvent. Filter sample through a 0.45 µm filter. Sample solution is prepared by Placing 10 µl of solution 1 onto a specific surface area of stainless steel plate. Swab the surface area; take the swab stick and place into a 10 ml volumetric flask, Add 10ml of solvent and sonicate for 10 minutes. Filter sample through a 0.45 µm filter. The precision will entail repeated testing of six samples prepared in the following manner: The % recovery should be greater than or equal to 65%. The analytical system complies with the requirements specified by the method precision.

Table: 4 Percentage of recovery

Sample	% Recovery
1	100
2	79
3	87
4	74
5	88
6	67
Mean	83

CONCLUSION

The method for the assay of Methyl Hydroxy benzoate complies with the requirements for specificity, system suitability, linearity, accuracy and method precision across the range of 50 % to 150 %. The method is therefore acceptable as valid and stability indicating.

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