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NEW VALIDATED METHOD FOR SIMULTANEOUS ANALYSIS OF METHYLPARABEN & PROPYLPARABEN IN POLYHERBAL FORMULATION (ORAL LIQUID DOSAGE FORM)

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ABSTRACT

An isocratic reversed phase high-performance liquid chromatographic (RP-HPLC) method has been developed for the simultaneous determination of methylparaben and propylparaben in poly herbal formulation at 254nm. Chromatographic separation was achieved on C18 WP, 100A column (250mm x 4.6 mm, 5 µm) column using mobile phase, methanol: water: acetonitrile (40:40:20 v/v/v) having flow rate of 1.2 mL min -1 at room temperature. Calibration curves were linear over range of 40 – 60.2 µg mL $^{-1}$ of methylparaben with a correlation coefficient ± 0.99 and 7.9 – 11.9 µg mL -1 of Propylparaben with coefficient correlation ± 0.99. Method is rapid, accurate, precise and specific for the routine quality control analysis of preservative content in poly herbal oral liquid dosage form.

Keywords: Poly Herbal Formulations, Oral Liquid, Simultaneous determination of MP & PP, Methylparaben, Propylparaben, HPLC.

INTRODUCTION

Methylparaben and Propylparaben are the preservatives widely used preservatives in cosmetics, food, beverages and pharmaceuticals[1] Both preservatives are collectively called as PARABENS. These compounds are used primarily for their bactericidal and fungicidal properties. [2] Structure of methylparaben and propylparaben is shown in figure 1: Literature survey revealed that number of publications those showed that many methods are available for simultaneous analysis of methylparaben and propylparaben in pharmaceutical products. Several RP-HPLC methods have been reported for the assay of various paraben in cosmetics and pharmaceutical products [3, 4, 5, 6] and gas chromatography (GC). [7, 8, 9] Method developed by S. Kumar, S. Mathkar, C. Romero and A.M. Rustum was the analytical method for simultaneous determination of four paraben namely methylparaben (MP), ethylparaben (EP), propylparaben (PP) and butylparaben (BP) and this method was developed for quick analysis of paraben in pharmaceutical and cosmetics products. [10] Another method developed by coral Barbas and Javeir Ruperze with gradient system for analysis of 4 paraben (MP, EP, and PP & BP) for food, beverages and cosmetics. [11] Work on simultaneous method was done by various researchers but specially performed for allopathic and cosmetics product, no specific method was developed for poly herbal dosage form. Although these method are also accurate, precise and reproducible but the mobile phases use are expensive and time consumption is high due to higher retention time. The objective of current study is to develop rapid, sensitive and cost effective method for simultaneous analysis in poly herbal formulation i.e. Syrup Dosage form using high Performance Chromatography for determination of Methylparaben and propylparaben and applying this method to analyze sample of oral liquid dosage form for routine quality control analysis.

EXPERIMENTAL

Reagent

Methylparaben Standard, Propylparaben standard,

Acetonitrile, Polyherbal Oral Liquid: Linkus Syrup B # 2613 148

Instrumentation
High Performance Liquid Chromatography: Dionex UHPLC provided with UV detector
Column: C18 WP, 100A (250mm x 4.6mm x 5.0µ)
Chromatographic Conditions
Mobile phase: Methanol:water:Acetonitrile (40:40:20 v/v/v)
Wavelength: 254nm
Flow rate: 1.2mL
Temperature: Ambient

Preparation of standard and sample solution
Preparation of standard solution:
Methylparaben: 50mg of standard were accurately weight and transfer into 100mL volumetric flask, dissolve in methanol and make up the volume with methanol and mix well.
Propylparaben: 10mg of propylparaben were accurately weight and transfer to 100mL volumetric flask, dissolve in methanol and make up the volume with methanol and mix well.

Working standard: 10mL of both standard transfers to 100mL volumetric flask through volumetric pipette and make up the volume with mobile phase and mix well.

Preparation of sample solution: Transfer 50mL of syrup to 100mL volumetric flask and dilute the content in methanol, mix well and make up the volume. Filter the solution with whattman filter # 40. Transfer 10mL to another 100mL volumetric flask, add mobile phase, mix and make up the volume with mobile phase.

RESULT

Method development and optimization: Separate injection of methylparaben and propyl paraben was run in triplicate to measure the retention time of both molecules with mobile phase system at wavelength 254nm and flow rate 1.2mL. Retention time found in individual chromatogram is 4-5 minutes and 9-10minutes respectively for methylparaben and propylparaben (injection volume 20µl). Chromatograms for individual run of methylparaben and propylparaben as well as combined working standard are shown in Figure 2.

System suitability: Five consecutive injections were run to check the system suitability. Results are summarized in TABLE I.

Specificity or Selectivity: Method specificity or selectivity was performed by running placebo samples with standard. There is no peak at same retention time of methylparaben and propylparaben peaks in standard run as shown in Figure 3.

Limit of Detection (LOD) & Limit of Quantitation (LOQ): The limit of detection (LOD) and limit of quantitation (LOQ) of this method were determined from the known concentrations of Methylparaben and Propylparaben. The LOD and LOQ for this analysis were calculated from using three and ten times the noise level of the response, respectively. Results are given in Table II.

Precision (Inter-day): Same samples were kept at ambient room temperature and again inject 5 consecutive run of same samples by using same chromatographic condition on lab alliance HPLC. Results are compared in Table III:

Linearity: Linearity is generally reported as the variance of the slope of the regression line. Linearity of analysis system was tested with known concentrations of methylparaben and propylparaben i.e. 40, 45, 50.1, 55, 60.2ug/mL and 7.9, 9.1, 10.2, 11, and 11.9µg/mL of methylparaben and Propylparaben respectively. Two injections were runs for every concentration. Injected concentrations versus area were plotted and the correlation coefficients were calculated which are shown in table IV.

Acceptance Criteria: $r^2 = 0.98 – 1.02$
ICH recommends that, for the establishment of linearity, a minimum of five concentrations normally be used. It is also recommended that the following minimum specified ranges should be considered:
Assay of a Drug Substance (or a finished product): from 80% to 120% of the test concentration. [12]

Application of method on poly herbal formulation: Results of the batch with this method has been compared with the already available method and found comparable. Results are shown in Table V.

DISCUSSION

The method was developed and optimized by injecting separate injection of methylparaben & propylparaben standard in triplicate with same solvent system i.e. Methanol:water:Acetonitrile (40:40:20 v/v/v) at 254nm. Based on the retention time a combine working standard was made and injected with same parameter and obtained reproducible results. The same has been confirmed

with the test for system suitability in which five consecutive injections of working standard were run. The method selectivity/specificity was performed by injecting standard and placebo samples and no peak were observed at the retention time of methylparaben and propylparaben. Test for Linearity was performed in the range 40 – 60.2 µg mL -1 of methylparaben with a correlation coefficient ± 0.99 and 7.9 – 11.9 µg mL -1 of Propylparaben with coefficient correlation ± 0.99. Results reveal that method is linear for the range of assay i.e. 80 – 120%. Method precision (inter-day) was checked by performing test on same samples and system on next day by using Lab alliance HPLC and results RSD found less than 2. A commercial batch of poly herbal product Linkus Syrup was randomly selected for analysis and method was applied to quantify the content of methylparaben and propylparaben in product.

CONCLUSION

A simple, rapid, sensitive and reliable HPLC method for simultaneous analysis of the methylparaben and propylparaben has been developed and method validity verified by performing system suitability, inter-day precision and linearity of the response. Proposed method is potential method for the utilization in analysis of commercial batches of poly herbal formulation (Oral Liquid Dosage Form).

TABLE I: SYSTEM SUITABILITY PARAMETERS

Material	Average Retention time	Average Area (mAU)	Standard deviation (SD)	Relative SD
Methylparaben	5.411	82.7054	1.31	1.58
Propylparaben	10.471	13.7252	0.12	0.88

TABLE II: LOD & LOQ RESULTS

Material	Concentration range	LOD µg/mL	LOQ µg/mL
Methylparaben	**40 – 60.2 µg/mL**	**0.028**	**0.085**
Propylparaben	**7.9 – 11.9 µg/mL**	**0.031**	**0.093**

TABLE III: INTER DAY PRECISION
RESULTS OF DAY 1

Material	Average Retention time	Average Area (mAU)	Relative SD
Methylparaben	5.411	82.7054	1.58
Propylparaben	10.471	13.7252	0.88

RESULTS OF DAY 2

Material	Average Retention time	Average Area (mV)	Relative SD
Methylparaben	4.734	4256.648	0.80
Propylparaben	9.626	7399.6	1.54

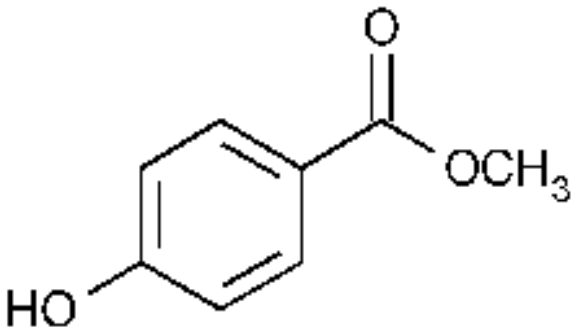
TABLE IV: RESULTS OF LINEARITY

Material	Concentration range	Correlation coefficient (r^2)
Methylparaben	**40 – 60 µg/mL**	**0.9930**
Propylparaben	**7.9 – 11.9 µg/mL**	**0.9958**

TABLE V: RESULTS COMPARISON

Material	Results		
	Old method	New method	RSD
Methylparaben	101.56% of label claim	102.92% of label claim	0.94
Propylparaben	99.5% of label claim	98.67% of label claim	0.59

Figure 1: Structure of methylparaben



Structure of Propylparaben

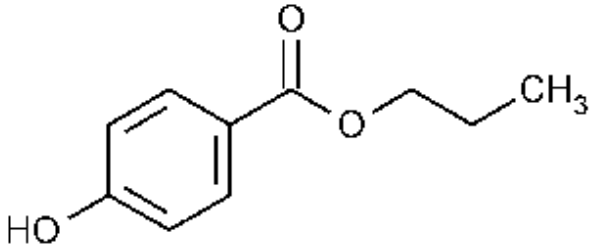


Figure 2: Chromatogram for methylparaben, propylparaben and working standard

Figure 2.1: Chromatogram of methylparaben standard Run

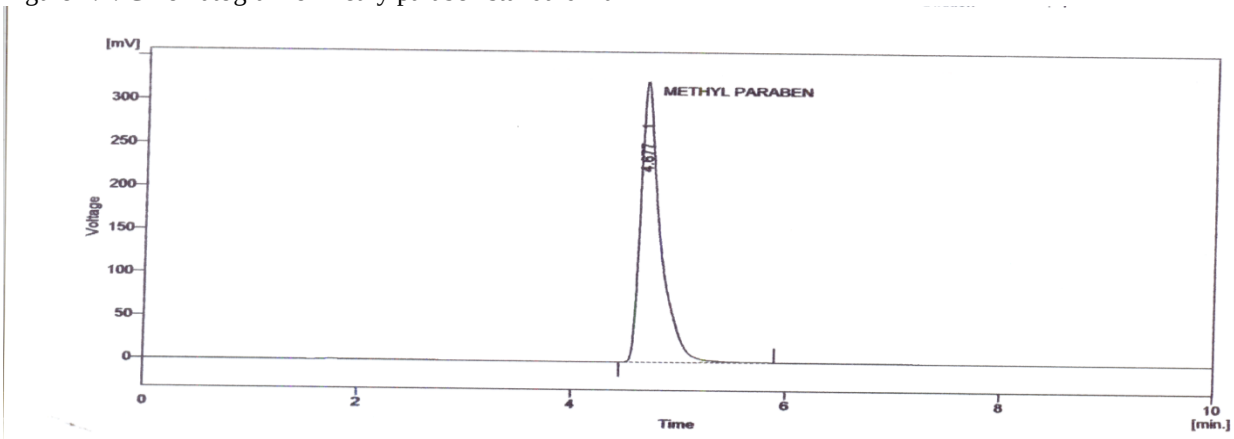


Figure 2.2: Chromatogram of Propylparaben standard Run

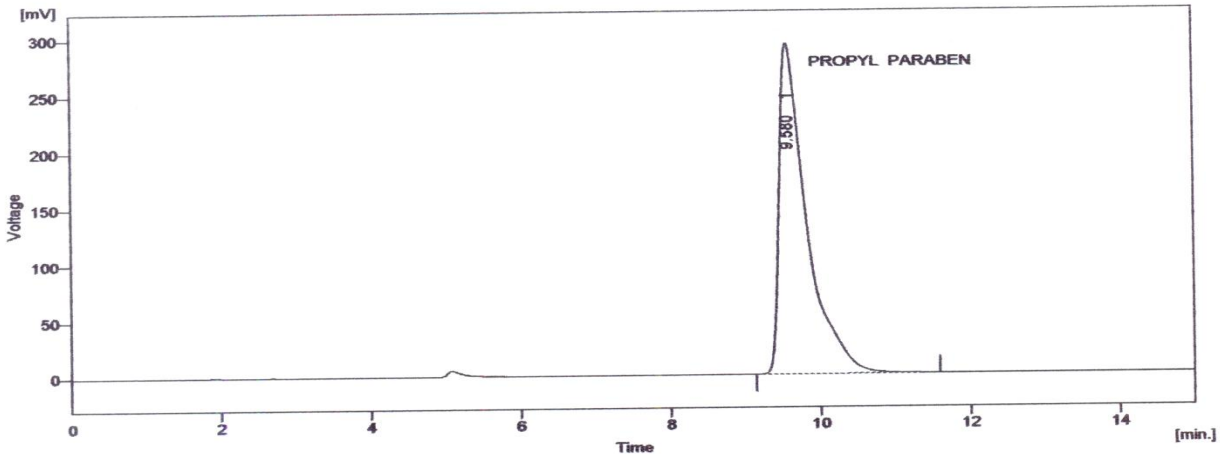


Figure 2.3: Chromatogram of combined working standard of methylpraben and Propylparaben

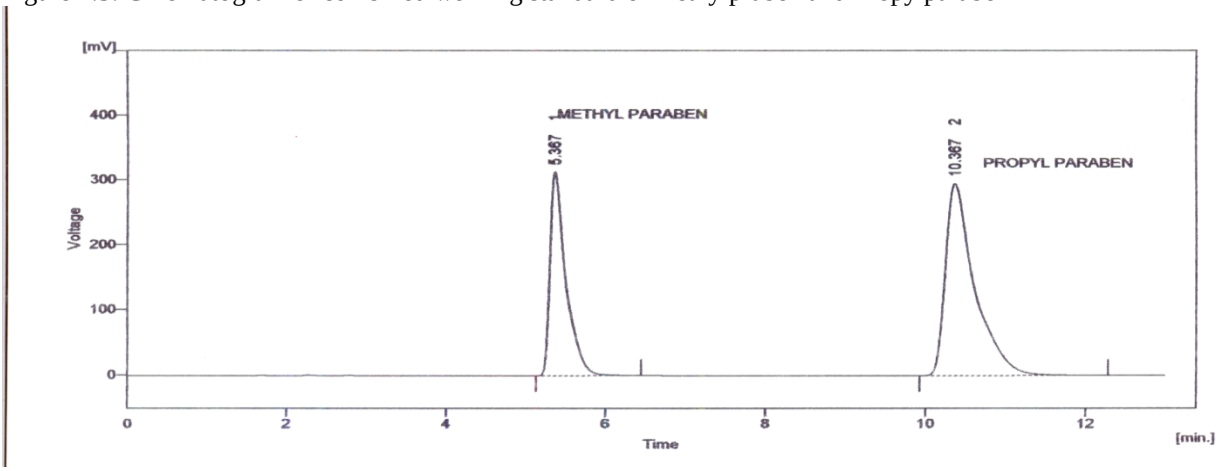


Figure 3: Standard of methylparaben and propylparaben & Placebo

Figure 3.1: Standard of Methylparaben & Propylparaben

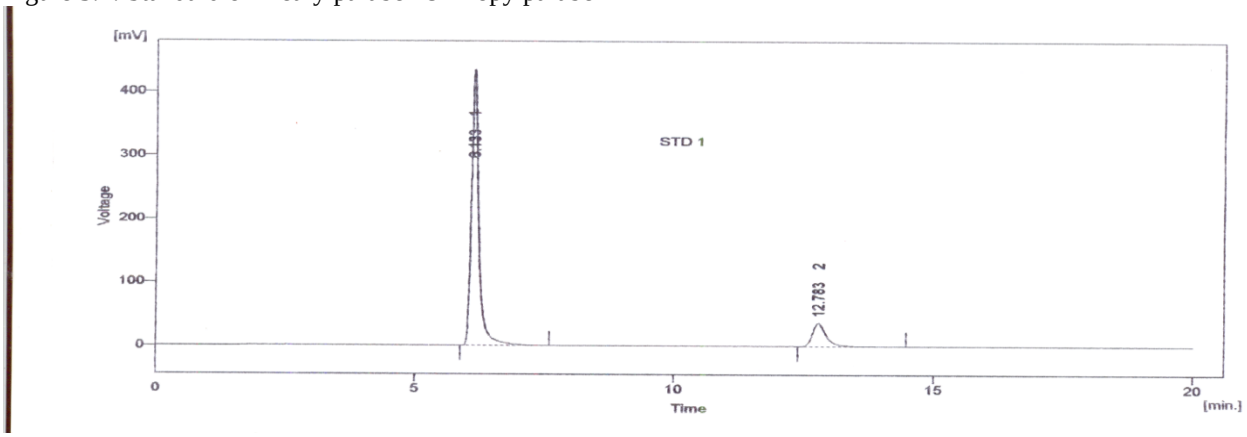
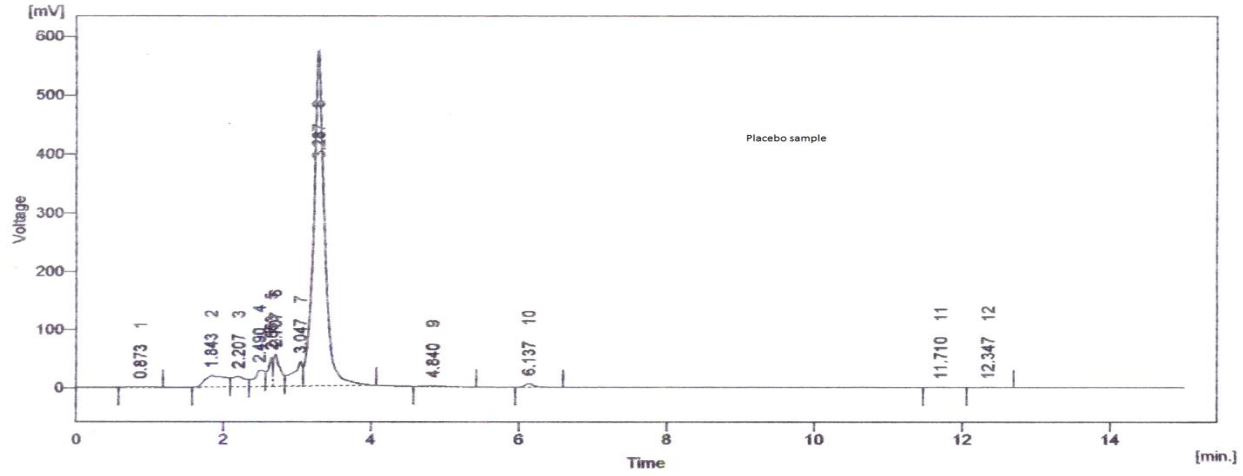


Figure 3.2: Placebo Sample



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