INTRODUCTION-

The quality is important in every product or service but it is more vital in medicine as it involve life. Analytical chemistry is mainly concerned about determining the qualitative and quantitative composition of material under study. Analytical monitoring of pharmaceutical product, or of specific ingredients within the product, is necessary to ensure its safety and efficacy throughout all phases of its shelf life, including storage, distribution and use.[1,2,3] Pharmaceutical analysis plays a very significant role in quality control of pharmaceuticals through a rigid check on raw material as well as finished product.[4] Analytical method development and validation play significant role in drug discovery, development, manufacturing of pharmaceuticals and estimation of small molecule in dosage form and biological system.[5] Method validation is the process used to confirm that analytical procedure employed for specific test is suitable for intended use.[6] Pharmaceutical chemistry is the chemistry of substances used in medicine and embrances organic, inorganic, analytical and radiochemistry.[7] Analysis is must to do task for all the research and development activities. It form the basis for quality, which is of prime concern regarding medicines in human life.[8] Pharmaceutical analysis helps in determination of the identity, strength, quality and purity of drug. Analytical chemistry is a subdivision of chemistry that has broad mission of understanding the chemical composition of all matter and developing the tools and experiments to make either qualitative or quantitative measurements.[9]

MATERIAL AND METHODS-

Analysis of standard drugs was done by following parameters:

- UV spectra and λ_{max}
- HPLC chromatogram and retention time

Selection of wavelength by UV-Visible Spectrophotometry:-

Preparation of standard stock solution:-

i. standard stock solution:

An accurately weighed quantity, 10 mg, of preservative was dissolved in methanol in 10ml volumetric flask and volume was made up to 10.0 ml to produce 1000 µg/ml.

Preparation of standard solution for Chromatogram:-

i. Preparation of Methyl paraben standard solution:

From the freshly prepared standard stock solution(1000µg/ml), 0.5 ml of stock solution was pipetted out in 10.0 ml of volumetric flask and volume was made upto 10 ml with mobile phase to get final concentration 50µg/ml.

Selection of mobile phase:

Mobile phase containing Acetonitrile and 0.05% orthro-phosphoric acid was selected since it gave sharp, well resolved peaks with symmetry within the limits and significant reproducible retention time for MP, PP.

Studies of Calibration plot:-

Optimzation of Chromatographic condition:

Column : C18 (150 mm× 4.6mm)

Particle size packing : 5µm

Detection wavelength : 254.0 nm and 210.0 nm

Flow rate : 0.7 ml/min

Temperature : Ambient

Sample size : 20 µl

Mobile phase : Acetonitrile and 0.05% orthro-phosphoric acid (60:40)

Procedure for calibration curve of Methyl paraben:

The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. From the freshly prepared standard stock solution(1000µg/ml), aliquots of 0.18, 0.36, 0.54, 0.72, 0.90 ml of stock solution were pipette out in 10.0 ml volumetric flask and volume was made upto 10.0 ml with mobile phase to get concentration of 18, 36, 54, 72, 90 µg/ml respectively. All samples were scanned for UV spectrum in the range 200-400 nm on UV

spectrophotometer.λ_{max} was traced and calibration curve of absorbance at 254 nm versus concentration of standard solution was constructed.

Procedure for calibration curve of Propyl paraben:

The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. From the freshly prepared standard stock solution(1000µg/ml), 1ml was pipetted out and dilute with 10.0 ml of mobile phase (100µg/ml). From it aliquots of 0.2, 0.4, 0.6, 0.8, 1.0 ml of stock solution were pipette out in 10.0 ml volumetric flask and volume was made upto 10.0 ml with mobile phase to get concentration of 2, 4, 6, 8, 10 µg/ml respectively. All samples were scanned for UV spectrum in the range 200-400 nm on UV spectrophotometer.λ_{max} was traced and calibration curve of absorbance at 254 nm versus concentration of standard solution was constructed.

Study of system suitability parameters:[12,13,14]

The system suitability is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The test was performed by collecting data from five replicate injections of standard solution.

Preparation of standard drug solution for chromatogram:

i. Mixed Standard Solution of Methyl paraben and Propylparaben:

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

Procedure:

The previously filtered mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. A 20 µl standard drug solution was injected and system suitability parameters were recorded for lab mixture of MP, PP, BAc and BKC.

Application of proposed method for estimation of MP and PP in Sterile dosage forms

a) Preparation of Standard Solution

Accurately weighed 10 mg of MP and 10 mg of PP were transferred to 10 ml separate volumetric flask and dissolved in methanol. The volume was made upto the mark. The standard stock solution of MP and PP were mixed and diluted properly with mobile phase to obtain a laboratory mixture containing 90 µg/ml of MP and 10 µg/ml of PP.

b) Preparation of sample solution

Accurately 1 ml of marketed sample solution(label claim-MP-0.18% w/v, PP-0.02% w/v) was transferred in 10 ml volumetric flask and dissolved in methanol(1800µg/ml). The solution was filtered through 0.45 u membrane filter and volume was made to the mark with mobile phase. Further 5 ml of solution were pipette out dilutions were made with the mobile phase to get final concentration of 90 µg/ml of sample solution.

Procedure:

Equal volumes (20µl) of standard and sample solutions were injected separately after equilibrium of stationary phase. The chromatograms were recorded and response i.e., peak area of major peaks were measured. The content of MP and PP was calculated by comparing a sample peak area with that of standard peak area.

RESULT AND DISCUSSION-

Verification of λ_{max} :

The solutions of MP, PP were scanned in the range of 200nm–400nm in 1cm cell against blank; from the spectrum wavelengths selected for the estimation of drugs were 254 nm as λ_{max} of Methyl paraben, Propyl paraben,.These are shown *i*n fig. 1.

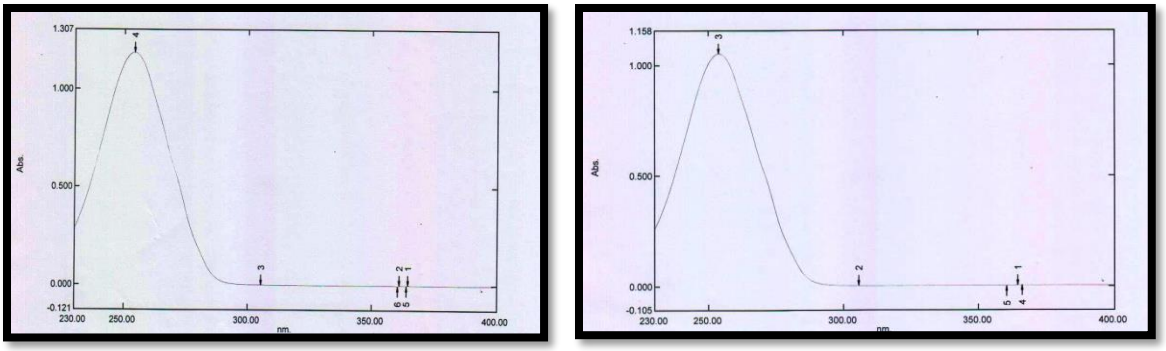


Fig. 1: UV Spectrum of Methyl parabenand Propyl parabenstandard

3. HPLC Chromatogram:

From the various mobile phases tried, mobile phase containing Acetonitrile and 0.05% ortho-phosphoric acid was selected since it gave sharp, well resolved peaks with symmetry within the limits and significant reproducible retention time for MP and PP. Chromatograms of MP and PP are shown in Fig .5 and 6.

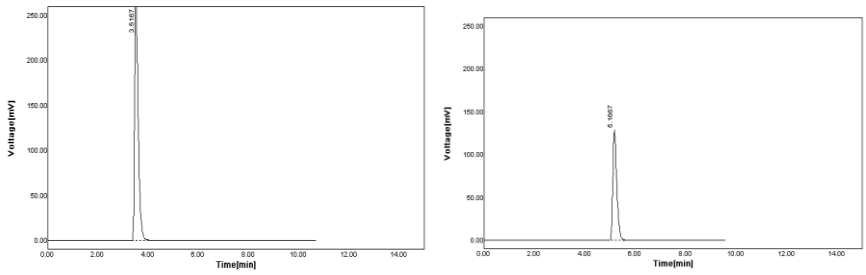
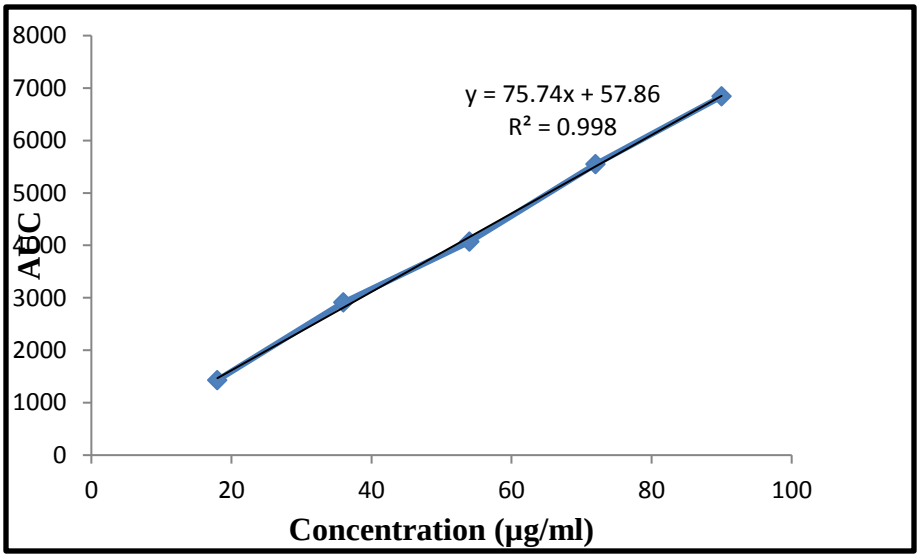


Fig. 5**: HPLC Chromatogram of MP standard with retention time 3.5 min.and PP 5.1 min.**

Calibration curve: The results of calibration curves of MP and PP are shown in Table no. 4 to 9 and in Fig. 9 to 12.

Sr. No.	Concentration(µg/ml)		Peak area(mv)	
	MP	PP	MP	PP
1	18	2	1429.8	114.10
2	36	4	2910.54	248.4
3	54	6	4067.78	410.4
4	72	8	5548.41	548.24
5	90	10	6840.77	690.80

Table 4: Standard calibration curve for MP and PP



(* Average of five replicates)

Fig. 9: Calibration curve of Methyl paraben

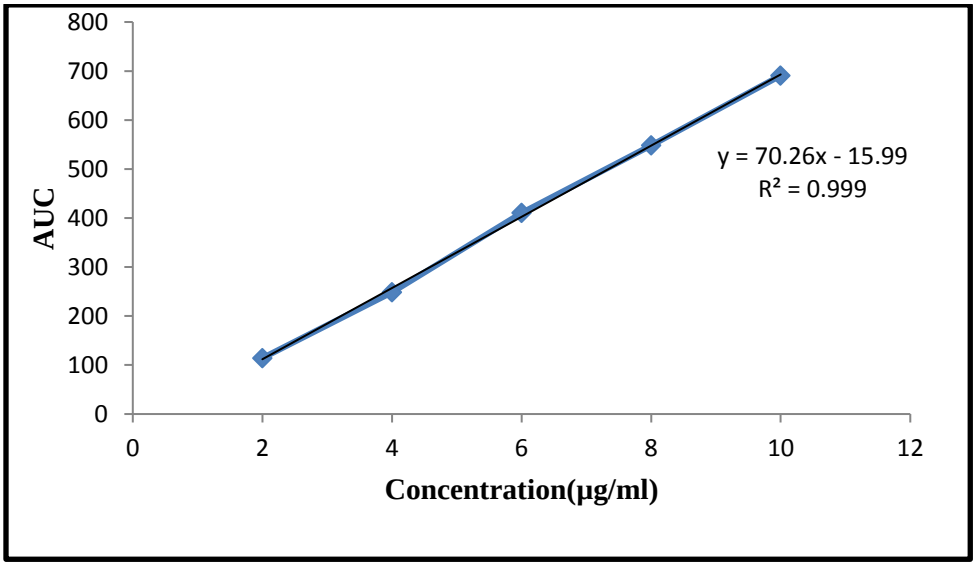


Fig. 10: Calibration curve of Propyl paraben

It was observed that, MP and PP obeys the Beer and Lambert's law. The regression coefficients were found to be 0.998 for MP, 0.999 for PP.

Study of System suitability parameter:

In study of system suitability parameter, retention time, asymmetry, no. of theoretical plate, capacity factor and resolution were calculated. These factors are found within limit. System suitability parameters were recorded for **lab mixture of MP, PP in Table 7, 8, 9 and fig. 13, 14, 15 respectively.**

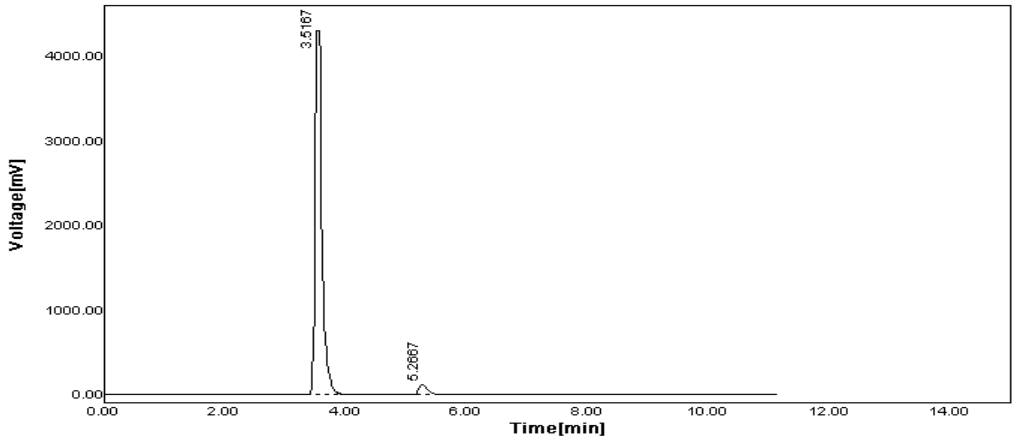


Fig. 13: Chromatogram obtained for standard MP and PP showing retention time of 3.5 and 5.2 min.

Table 7 : Result for System suitability parameter of mixture of MP and PP

Sr. No	Retention time(min)		Assymetry		No.of theorotical plate		Capacity factor		Area under curve(mv)		Resolution
	MP	PP	MP	PP	MP	PP	MP	PP	MP	PP	
1	3.5	5.2	1.33	1.21	4863.3	6851.7	0.52	1.05	6840.1987	690.80	3.2
2	3.5	5.2	1.33	1.21	4862.8	6859.6	0.54	1.05	6840.2853	690.48	3.7
3	3.4	5.2	1.34	1.26	4860.1	6852.5	0.53	1.02	6841.0558	690.89	3.6
4	3.5	5.3	1.38	1.27	4867.2	6849.8	0.53	1.03	6840.0480	687.98	3.3
5	3.5	5.2	1.36	1.24	4865.8	6857.3	0.52	1.05	6840.0685	688.47	3.5
							Mean		6840.33	689.92	
							±SD		0.3725	1.0014	
							%RSD		0.00545	0.1451	

Application of proposed method for estimation of mixture of MP and PP in Sterile dosage forms

For marketed sterile formulations, % drug estimation was calculated and % RSD were found to be 0.0024 for MP and 0.058 for PP. The % RSD were within limit.

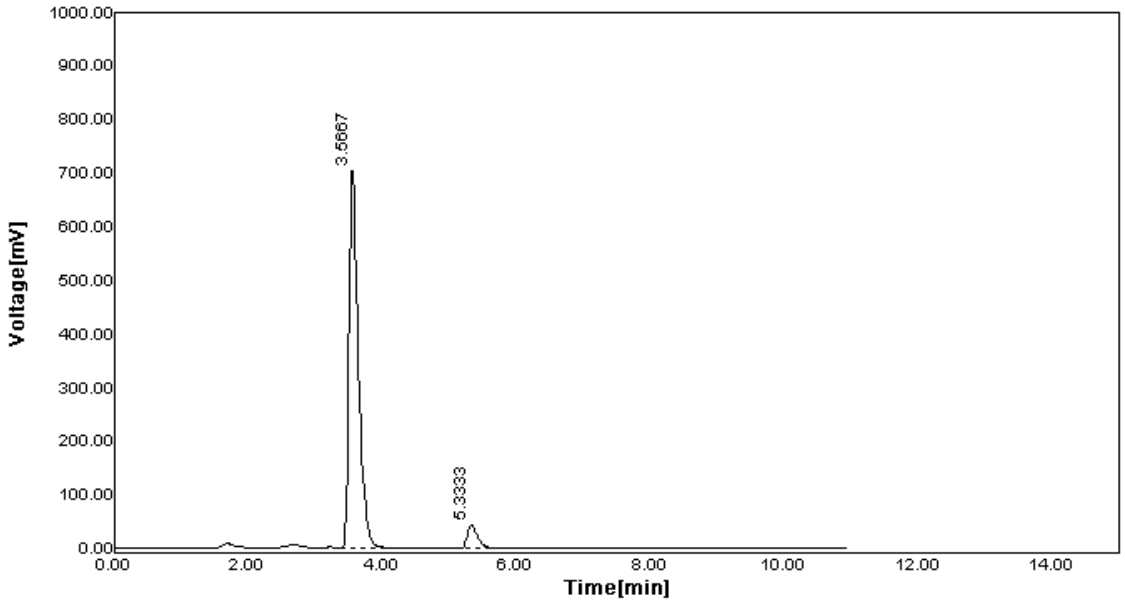


Fig. 16: Chromatogram of Sterile Marketed preparation of MP, PP showing retention time of MP= 3.5 min. and PP= 5.3 min.

Table 10: Result for estimation of mixture of MP and PP in Sterile formulation

Sr. No.	Conc. Of std.(µg/ml)		Conc. Of sample(µg/ml)		Peak area of std.(mv)		Peak area of sample(mv)		% Drug estimation	
	MP	PP	MP	PP	MP	PP	MP	PP	MP	PP
1	90	10	90	10	6840.2853	690.80	6840.7213	690.90	100.00	100.01
2							6840.2971	691.01	100.00	100.03
3							6840.4819	690.89	100.00	100.01
4							6840.9043	691.54	100.00	100.10
5							6840.8947	691.92	100.00	100.16
								Mean	100.00	100.06
								±SD	0.0024	0.059
								%RSD	0.0024	0.058

7.7. Validation

i. Accuracy:

The accuracy of the proposed method was evaluated by performing recovery studies and the % RSD and % recovery were within the acceptable limits in all 3 levels and % recovery was found to be 100.52 % for Methyl paraben, 98.65 % for Propyl paraben,99.85% for Benzyl alcohol and 100.79% for Benzalkonium chloride. It is evident from the results of accuracy that the proposed method is very accurate.

Table 13: Results and statistical data for recovery study of mixture of MP and PP.

Sr.No.	Conc.(µg/ml)		Peak area of std.(mv)		Amount of pure drug added (µg/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

Table 7 : Result for System suitability parameter of mixture of MP and PP

Sr. No	Retention time(min)		Assymetry		No.of theorotical plate		Capacity factor		Area under curve(mv)		Resolution
	MP	PP	MP	PP	MP	PP	MP	PP	MP	PP	
1	3.5	5.2	1.33	1.21	4863.3	6851.7	0.52	1.05	6840.1987	690.80	3.2
2	3.5	5.2	1.33	1.21	4862.8	6859.6	0.54	1.05	6840.2853	690.48	3.7
3	3.4	5.2	1.34	1.26	4860.1	6852.5	0.53	1.02	6841.0558	690.89	3.6
4	3.5	5.3	1.38	1.27	4867.2	6849.8	0.53	1.03	6840.0480	687.98	3.3
5	3.5	5.2	1.36	1.24	4865.8	6857.3	0.52	1.05	6840.0685	688.47	3.5
							Mean		6840.33	689.92	
							±SD		0.3725	1.0014	
							%RSD		0.00545	0.1451	

ii. Precision:

The % RSD of method precision were found to be 0.0062 for Methyl paraben, 0.0754 for Propyl paraben. The % RSD below 2.0 shows high precision of proposed method

Table 16: Results of precision for mixture of MP and PP

Sr.No.	Conc.(µg/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	98.85
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

.

a. Intraday

It was performed by using same procedure as under marketed formulation analysis and peak area was recorded at 3 h interval within a day. The % label claim was calculated by using same formula as for marketed for formulation analysis. Results and statistical data are shown in Table 22, 23 and 24 respectively.

Table 19: Result of Intraday studies of mixture of MP and PP

Sr. No.	Conc.(µg/ml)		Peak area of std.(mv)		Peak area of sample(mv)		% Label claim	
			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	98.97
						±SD	0.008165	0.0216
						%RSD	0.00806	0.2180

a. Interday (Different days)

It was also performed by using same procedure as under marketed formulation analysis and peak area of same sample was recorded on different days. The % label claim was calculated using same formula as for marketed formulation analysis. Results and statistical data are shown in Table 25, 26 and 27 respectively.

Table 22: Result of Interday studies of mixture of MP and PP

Sr. No.	Conc.(µg/ml)		Peak area of std.(mv)		Peak area of sample(mv)		% Label claim	
	MP	**PP**	**MP**	**PP**	**MP**	**PP**	**MP**	**PP**
Day 1	90	10	6840.7853	691.80	6840.9852	690.80	101.20	98.98
Day 2					6842.1823	693.52	101.22	99.20
Day 3					6843.5461	695.72	101.24	99.49
						Mean	101.22	99.22
						±SD	0.01613	0.2083

%RSD	0.01613	0.2104

B. Different Analysts:

The sample solution was prepared by three different analysts and same procedure was followed as described earlier. The %labeled claim was calculated as done in marketed sterile formulation estimation. This is shown in Table25, 26 and 27 respectively.

Table 25: Result of Different analyst study for mixture of MP and PP

Analyst	**Conc.(µg/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	101.22	99.19
						±SD	0.0047	0.0509
						%RSD	0.0046	0.0514

v. Linearity and range:

Accurately measured quantities of sterile solution equivalent to 80, 90, 100, 110 and 120 % of label claim were taken and dilutions were done appropriately to obtain a concentration in the range of 80-120% of the test concentration and absorbance wererecorded at 254 nm for MP, PP, BAc and 210 nm for BKC. These are shown in fig. 19, 20, 21 and 22 respectively.

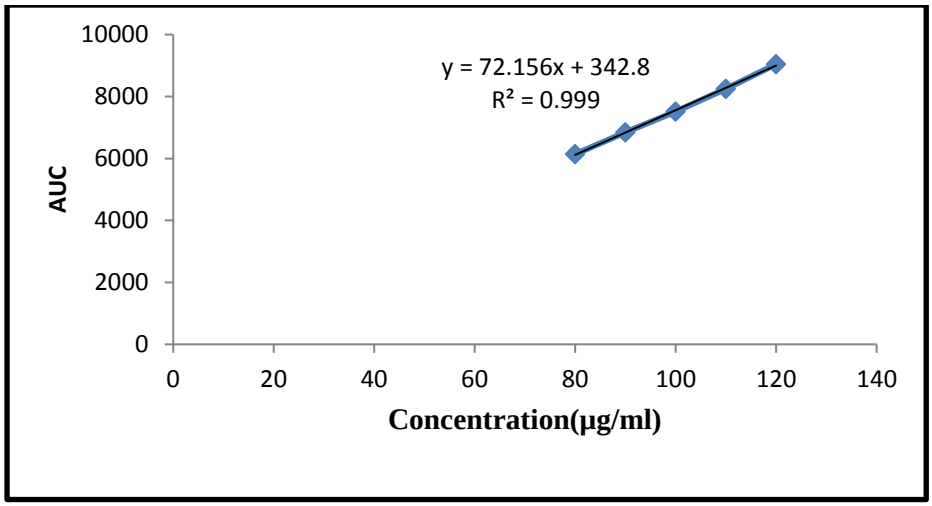


Fig. 19: The plot of Linearity and range study for Methyl paraben

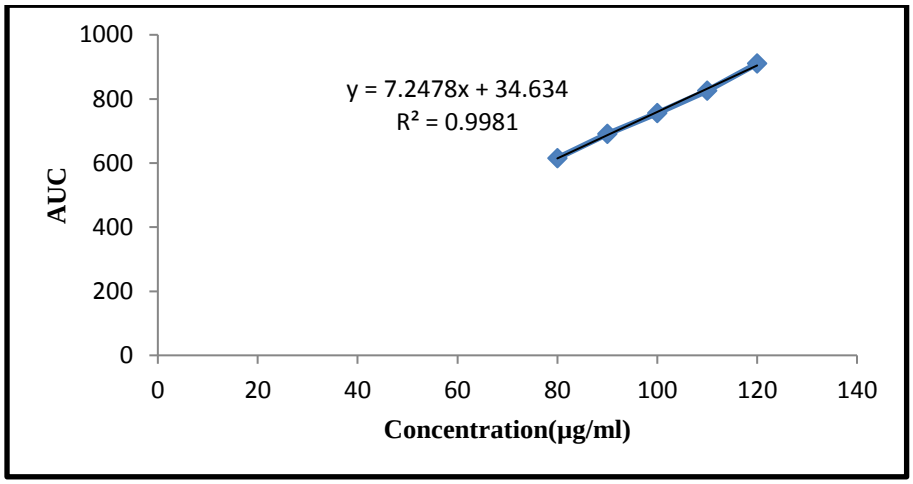


Fig. 20: The plot of Linearity and range study for Propyl paraben

It was observed that, MP and PP obeys the Beer Lambert's law and the regression coefficients were found to be 0.999 for MP, 0.998 for PP.

vi. LOD and LOQ:

The LOD and LOQ for Methyl paraben was found to be 0.016µg/mL and 0.049 µg/mL, for Propyl paraben, 0.047 µg/mL These are shown in table 28.

Table28: Result of LOD and LOQ

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

8.

SUMMARY AND CONCLUSION:

8.1. Summary:

The present research study was carried out with an aim to develop and validate HPLC method for determination of Preservatives in sterile dosage forms.

The isobestic point was selected 254 nm for Methyl parben andPropyl paraben.

The method is selective and linear between concentration range of 18-90 µg/mL, 2-10 µg/mL for Methyl paraben, Propyl parabenrespectively.

The mobile phase was developed after trial and error and selected Acetonitrile:0.05% O-phosphoric acid(60:40) was selected to get a well separated peaks of pure and marketed sample of Methyl paraben and Propyl paraben. The retention time of Methyl paraben, Propyl paraben, was found 3.5, 5.2, min. respectively.

A system sutability parameter was developed on every day basis which includes number of theoretical plate, resolution ,Tailing factor, Area under curve were found within the limits.

Results of estimation of Methyl paraben, Propyl paraben, in sterile formulation selected for study were found in range 98.65% -100.79% that is Accurate , precise and with % RSD below 2.

The developed method was validated by means of accuracy, precision, linearity, ruggedness, LOD and LOQ as per ICH guaideline and were found within limit.

Moreover, LOD and LOQ for Methyl parabenwas found to be 0.016µg/mL and 0.049 µg/mL, for Propyl paraben, respectively. Thus the method is specific and sensitive. Statistical analysis

proves that the method is suitable for the analysis of Methyl paraben, Propyl paraben, as preservatives in sterile dosage forms.

CONCLUSION-

The present research study was carried out with an aim to develop and validate HPLC method for determination of Preservatives in sterile dosage forms.Selection of wavelength was done by scanning the standard Methyl paraben, Propyl paraben, solution (10μg/ml) on shimandzu UV-visible spectrophotometer. The isobestic point was selected 254 nm for Methyl parben, Propyl paraben.The method is selective and linear between concentration range of 18-90 µg/mL, 2-10 µg/mL for Methyl paraben, Propyl paraben respectively. The mobile phase Acetonitrile: 0.05% O-phosphoric acid(60:40) was selected to get a well separated peaks of pure and marketed sample of Methyl paraben, Propyl paraben, The retention time of Methyl paraben, Propyl paraben, was found 3.5, 5.2, min. respectively.A system sutablity parameter was developed on every day basis which includes number of theoretical plate, resolution ,Tailing factor, Area under curve were found within the limits.Results of estimation of Methyl paraben, Propyl paraben, in sterile formulation selected for study were found in range 98.65% -100.79% that is Accurate , precise and with % RSD below 2.The developed method was validated by means of accuracy, precision, linearity, ruggedness, LOD and LOQ as per ICH guaideline and were found within limit. Moreover, LOD and LOQ for Methyl parabenwas found to be 0.016 µg/mL and 0.049 µg/mL, for Propyl paraben, respectively. Thus the method is specific and sensitive. Statistical analysis proves that the method is suitable for the analysis of Methyl paraben, Propyl paraben as preservatives in sterile dosage forms.

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