WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Analytical Method Development and Validation of Preservatives by Using RP-HPLC in Suspension Dosage Form

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ABSTRACT:

To develop and validate high performance liquid chromatographic method for determination of preservatives in pharmaceutical suspension dosage form NEED &OBJECTIVE: The objective of validation of analytical procedure is to demonstrate that it is suitable for its intended purpose. Any developed method may be influenced by variables like different elapsed assay times, different days, reagents lots, instruments, equipment's, environmental conditions like temperature, etc so it is expected that after the method has been developed and before it is communicated or transferred from one lab to the other, it is properly validated and the result of validity tests reported. For analytical method validation of pharmaceuticals, guidelines from the International Conference on Harmonization (ICH), United States Food and Drug Administration (US FDA), American Association of Official Analytical Chemists (AOAC)United States Pharmacopoeia (USP), and International Union of Pure and Applied Chemists (IUPAC) provide a framework for performing such validations in a more efficient and productive manner. The primary objective of validation is to form a basis for written procedure for production and process control which are designed to assure that the drug products have the identity, strength, quality and purity they purport or are represented to possess quality, safety and efficacy must be designed to build into the product. Each step of the manufacturing process must be controlled to maximize the probability that the finished products meet all quality and design specification The objective of the present work is develop analytical method and to validate for preservative assay of Fasimec suspension (Methyl paraben, Propyl paraben and Benzyl Alcohol) and Fasinex suspension & Endex suspension (Sodium Methyl Paraben) that the method consistently yields the results which reflects the quality characteristics of the product Validation of developed method as per ICH guidelines.

Keyword: Analytical Method, Validation, Rp-Hplc, Suspension Dosage Form, Methyl Paraben

INTRODUCTION

Analytical Method Development Analytical method development and validation are key elements of any pharmaceutical development program. HPLC analysis method is developed to identify, quantity or purifying compounds of interest. This technical brief will focus on development and validation activities as applied to drug products [1,2]. When there are no authoritative methods are available, new methods are being developed for analysis of novel products. To analyze the existing either pharmacopoeia or nonpharmacopoeia products novel methods are developed to reduce the cost besides time for better precision and ruggedness. These methods are optimized and validated through trial runs. Alternate methods are proposed and put into practice to replace the existing procedure in the comparative laboratory data with all available merits and demerits [1,2]. 1.1.1Purpose of analytical method development Drug analysis reveals the identification characterization & determination of the drugs in mixtures like dosage forms & biological fluids. During manufacturing process and drug development the main purpose of analytical methods is to provide information about potency (which can be directly related to the requirement of a known dose), impurity (related to safety profile of the drug), bioavailability (includes key drug characteristics such as crystal form, drug uniformity and drug release), stability (which indicates the Errors at varied stages in production. To take a decision to release or discard a product is based on one or more sorts of control actions. Providing simple and analytical process for various complex formulations is a subject matter of utmost importance. Rapid increase in pharmaceutical industries and constant production of drug in various parts of the world has brought a quick rise in demand for new analytical techniques in the pharmaceutical industries as a consequence; analytical method development has become the basic activity of analysis in a quality control laboratory. The reasons for the development of novel methods of drug analysis are: a) When there is no official drug or drug combination available in the pharmacopoeias. b) When there is no decorous analytical process for the existing drug in the literature due to patent regulations. c) When there are no analytical methods for the formulation of the drug due to the interference caused by the formulation excipients.

EXPERIMENTAL WORK

Preparation of solutions:

Preparation of standard stock solution of preservatives Fasimec Suspension:

Standard stock solution of it was prepared by weighing 55 mg of Methyl Paraben, 20 mg of Propyl Paraben and 100 mg of Benzyl Alcohol into a clean and dry 100 ml of volumetric flask..

Fasinex and Endex Suspension:

Standard stock solution of it was prepared by weighing 7.5 mg of Sodium Methyl Paraben into

a clean and dry 100 ml of volumetric flask. Then 70 ml of Methanol was added and sonicated for 5 minutes, shaked well and kept the flask for 10 minutes at Room Temperature and the volume was made upto

100 mlwith Methanol and mixed well. Further 10 ml of above Solution diluted into a clean and dry 100 ml of volumetric flask made up the volume with Methanol and mixed well.

Preparation of Sample Solutions:

Fasimec Suspension:

Sample solution of Fasimec Suspension was prepared by weighing accurately 5 g of Sample into a clean and dry 100 ml of volumetric flask. Then 70 ml of Methanol was added and sonicated for 10 minutes, shake well and kept the flask for 10 minutes at Room Temperature and the volume was made upto 100 ml the with Methanol and mixed well. The Sample was collected in a HPLC vial by passing through 0.2 um PTFE filter, before making injection into the HPLC system.

Fasinex Suspension:

Sample solution of Fasinex Suspension was prepared by weighing accurately 5.150 g of Sample into a clean and dry 100 ml of volumetric flask. Then 70 ml of Methanol was added andsonicated for 10 minutes, shaked well and kept the flask for 10 minutes at Room Temperature and the volume was made upto 100 ml thewith Methanol and mixd well. The Sample was collected in a HPLC vial by passing through 0.2 um PTFEfilter, before making injection into the HPLC system

RESULTS AND DISCUSSION

HPLC method for Preservatives used in Fasimec Suspension

Various trials for method development were done representative chromatograms were presented in figure Nos.

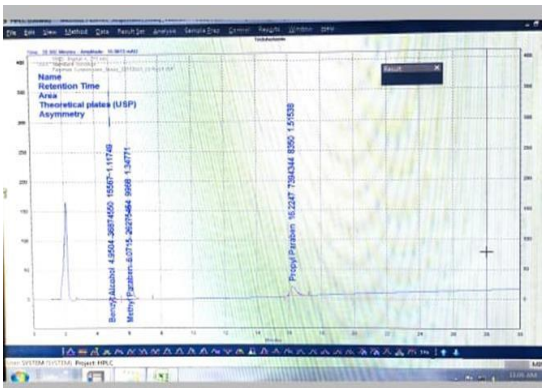

Fig No. chromatogram trial 1

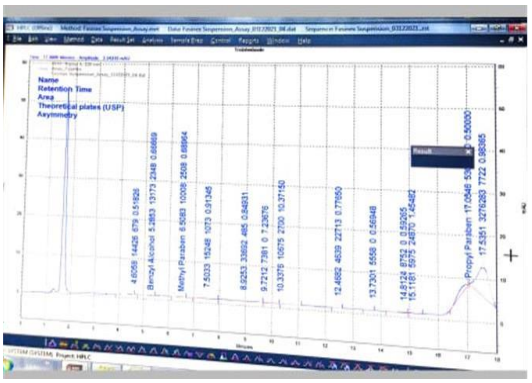

Fig No.: chromatogram trial 2

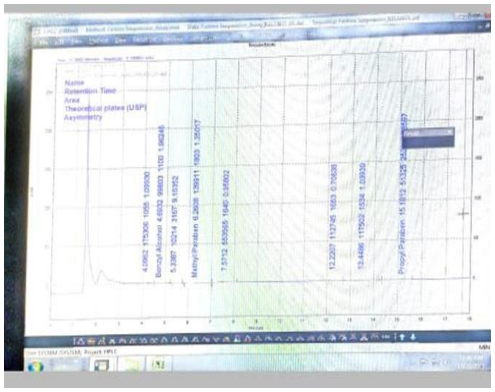

Fig No.: chromatogram trial 3

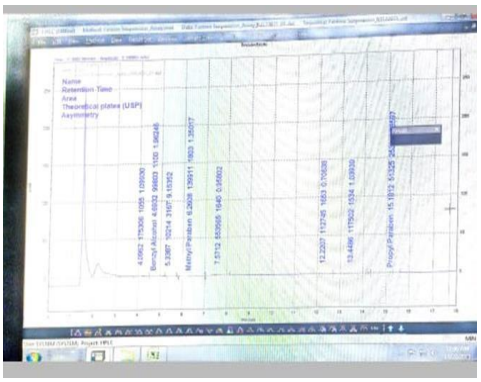

Fig No.: chromatogram trial 3

Table : Optimized chromatographic conditions for HPLC method

Mobile phase	Mobile Plase A: Water Mobile Phase B: Acetonitrile
Column	C18 (250 mm× 4.6mm)
Particle size packing	5µm
Flow rate	1.2 ml/min
Detector.	UV
Detection wavelength	215 nm
Temperature	Ambient
Sample size	10 µl
Diluent	Methanol

Validation of Analytical Method:

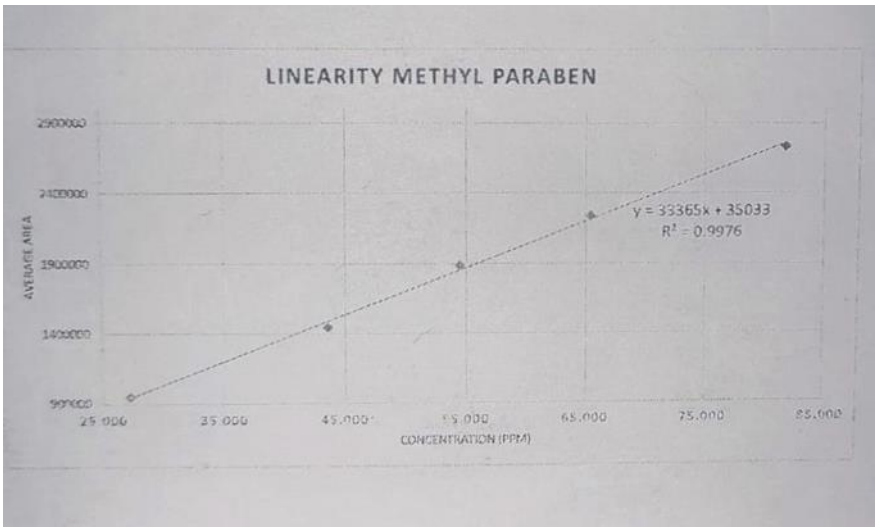

Fig . Calibration Curve for Methyl Paraben

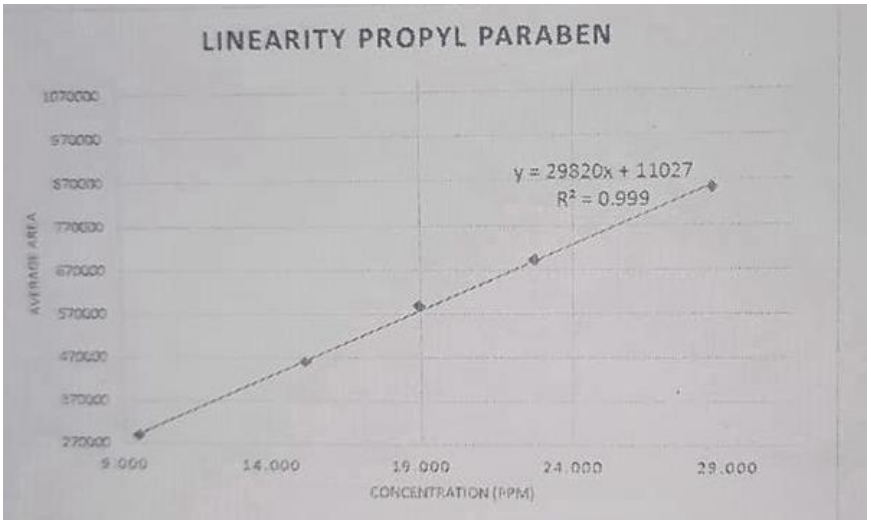

Fig No: Calibration Curve for Propyl Paraben

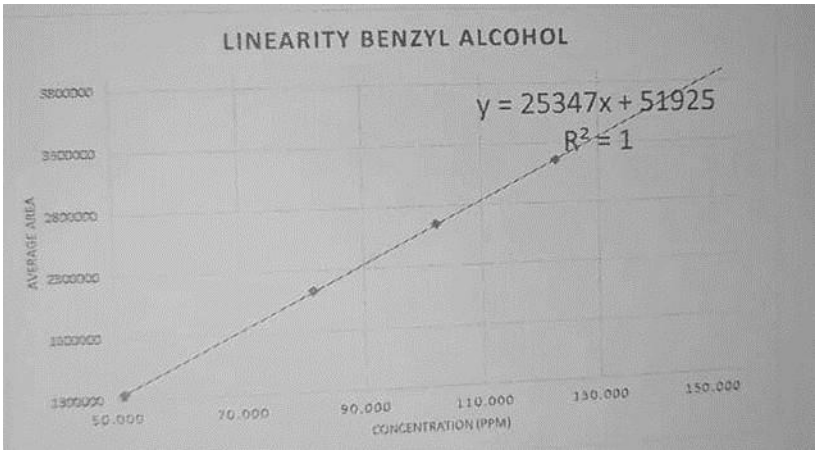

Fig No.: Calibration Curve for Benzyl Alcohol

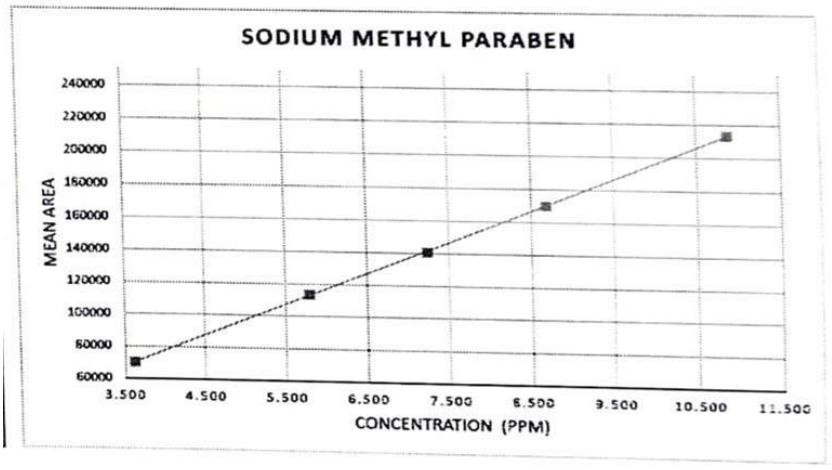

Fig No.: Calibration Curve for Sodium Methyl Paraben

Table No: Linearity Result for Methyl Paraben

% Level	Actual Conc. PPM	Peak Area	Mean Area	Std Dev	% RSD
50	27.231	946534	946646	146384	0.18
		946591			
		946812			
80	43.569	1461520	1445683	13731.02	0.95
		1437112			
		1438416			
100	54.462	1891442	1889790	1661.07	0.09
		1888120			
		1889807			
120	65.354	2243407	2243232	1356.46	0.06
		2244493			
		2241797			
150	81.693	2740537	2735506	4885.61	0.18
		2735202			
		2730780			
Correlation Coefficient		0.9988	NLT 0.99%		

Table No: Linearity Result for Propyl Paraben

% Level	Actual Conc. PPM	Peak Area	Mean Area	Std Dev	% RSD
50	9.480	289728	289941	489.23	0.17
		290501			
		289595			
80	15.168	463607	460052	3085.91	0.67
		458485			
		458064			
100	18.961	588138	587268	1942.11	0.33
		588623			
		585043			
120	22.753	693942	691788	1867.18	0.27
		690799			
		690624			
150	28.441	857774	853118	4049.52	0.47
		851164			
		850416			
Correlation Coefficient		0.9995	NLT 0.99%		

Table No: Linearity Result for Benzyl Alcohol

% Level	Actual Conc. PPM	Peak Area	Mean Area	Std Dev	% RSD
50	51.099	1339627	1340527	787.72	0.06
		1340863			
		1341091			
80	81.758	2147778	2131818	13820.23	0.65
		2124033			
		2123647			
100	102.198	2651706	26484992	2895.40	0.11
		2647681			
		2646088			
120	122.637	3141822	3154699	23528.53	0.75
		3181855			
		3140419			
150	153.297	3931916	3936285	43257.77	1.10
		3895377			
		3981561			
Correlation Coefficient		1.0000	NLT 0.99%		

Table No: Linearity Result for Sodium Methyl Paraben

% Level	Actual Conc. PPM	Peak Area	Mean Area	Std Dev	% RSD
50	3.620	70383	70252	1179.50	1.68
		69012			
		71360			
80	5.792	112731	113399	633.49	0.56
		113991			
		113476			
100	7.240	140906	140802	297.94	0.21
		140466			
		141034			
120	8.688	170005	170064	74.14	0.04
		170039			
		170147			
150	10.860	213586	213499	88.02	0.04
		213410			

		213501			
Correlation Coefficient		1.0000	NLT 0.99%		

Table No : Linear regression data for calibration curves

Parameters	**Methyl Paraben**	**Propyl Paraben**	**Benzyl Alcohol**	**Sodium Methyl Paraben**
Linearity Range	27.231 -81.693	9.480 – 28.441	51.099 – 153.297	3.620 – 10.860
r2	0.9976	0.9995	1	1
Slope	33365	29820	25347	19496
Y Intercept	-0.91	-0.35	-2.0	1425.4
Y=mX +C	y = 33365x + 35033	y=29820x + 11027	y= 25347 +51925	y= 19496x + 1425.4

Limit of Detection and Limit of Quantitation:

The LOD and LOQ values for methyl paraben, propyl paraben, benzyl alcohol and sodium methyl paraben was found to be as following:

Table No: 8.7 LOD & LOQ Values

Preservatives	**LOD**	**LOQ**
Methyl Paraben	0.4308	1.3056
Propyl Paraben	0.2530	0.7668
Benzyl Alcohol	2.1947	6.65
Sodium Methyl Paraben	0.0769	0.2331

Precision

Results of System precision and Method precision of Methyl Paraben, Propyl Paraben, Benzyl Alcohol and Sodium Methyl Paraben was found to be as per following:

Table No:8.8 System precision for MP, PP and BA

Statistics	MP		PP		BA	
	RT	**Peak area**	**RT**	**Peak area**	**RT**	**Peak area**
	6.1	1720270	15.7	502276	4.9	2374177
	6.1	1715811	15.7	502115	4.9	2371122
	6.1	1717807	15.7	501885	4.9	2373462
	6.1	1718368	15.7	501999	4.9	2370261
	6.1	1710992	15.7	505763	4.9	2363379
	6.1	1708199	15.7	503055	4.9	2359944
Average		1715241		502849		2368724
St. Dev		4682.35		1486.79		5761.15
%RSD		0.27		0.30		0.24

Table No: System precision for Sodium Methyl Paraben

Statistics	Sodium Methyl Paraben	
	RT	**peak area**
	5.8	2388222
	5.8	2394704
	5.8	2370616
	5.8	2383440
	5.8	2365463
	5.8	2364561
Average	5.8	2377834
St. Dev	0.01	12690.53
% RSD	0.13	0.53

Table No: Method precision for MP, PP and BA

Statistics	MP		PP		BA	
	RT	**Peak area**	**RT**	**Peak area**	**RT**	**Peak area**
	6.1	1754987	15.6	483655	4.9	216791
	6.1	1728177	15.6	488174	4.9	2141460
	6.1	1742045	15.6	483735	4.9	2152828
	6.1	1742894	15.6	478488	4.9	2155196
	6.1	1751537	15.6	480263	4.9	2162685
	6.1	1754198	15.6	489744	4.9	2165369
Average		1745640		484010		2157505
St. Dev		10205.89		4357.79		9726.69
% RSD		0.58		0.90		0.45

Table No: Method precision for Sodium Methyl Paraben

Statistics	Sodium Methyl Paraben	
	RT	**Peak area**
	6.3	136808
	6.3	140087
	6.3	137369
	6.3	137139
	6.3	136429
	6.3	137723
Average	6.3	137593
St. Dev	0.09	1301.10
% RSD	1.46	0.95

Robustness:

To obtain robustness by compairing %RSD caused due to small deliberate change in flow rate and wavelength. Thus results of robustness were found to be as per following

Table No. Robustness for MP, PP and BA, Flow rate: 1.1 ml/min

Statistics	MP		PP		BA	
	RT	**peak area**	**RT**	**peak area**	**RT**	**peak area**
	6.8	1998809	16.9	619729	5.4	2488560
	6.8	2001736	17.0	621593	5.4	2493875
	6.8	1988713	16.9	617183	5.4	2480510
	6.8	1985522	16.9	616335	5.4	2478068
	6.8	1986138	16.9	617650	5.4	2480324
Average	6.8	1992184	16.9	618498	5.4	2484267
St. Dev		7551.747		2134.32		6686.430
% RSD		0.38		0.35		0.27

Table No: Robustness for MP, PP and BA, Flow rate: 1.3 ml/min

Statistics	MP		PP		BA	
	RT	**Peak area**	**RT**	**Peak area**	**RT**	**Peak area**
	5.8	1672555	14.9	503662	4.6	2097866
	5.8	1672260	14.9	504222	4.6	2097777
	5.8	1671809	14.9	504970	4.6	2098255
	5.8	1670908	14.9	504977	4.6	2097667
	5.8	1669974	14.9	505568	4.6	2098091
Average	5.8	1671517	14.9	504682	4.6	2897913
St. Dev		1045.878		748.275		226.897
% RSD		0.96		0.15		0.91

Table No. Robustness for MP, PP and BA, Wavelength 213 nm

Statistics	MP		PP		BA	
	RT	**Peak area**	**RT**	**peak area**	**RT**	**Peak area**
	5.7	1932294	14.9	582390	4.6	2556983
	5.7	1931507	14.9	583236	4.6	2557268
	5.7	1931833	14.9	584571	4.6	2557661
	5.7	1930619	14.9	584189	4.6	2556907
	5.7	1931462	14.9	584981	4.6	2558103
Average	5.7	1931543	14.9	583873	4.6	2557384
St. Dev		614.356		1051.164		498.868
% RSD		0.03		0.18		0.02

Table No. Robustness for MP, PP and BA, Wavelength 217 nm

Statistics	MP		PP		BA	
	RT	**peak area**	**RT**	**Peak area**	**RT**	**Peak area**
	5.7	1263495	14.8	382375	4.6	1437154
	5.7	1262967	14.8	383249	4.6	1437486
	5.7	1262850	14.8	383013	4.6	1437109
	5.7	1262288	14.8	382867	4.6	1436723
	5.7	1263394	14.8	384027	4.6	1438314
Average	5.7	1262999	14.8	383106	4.6	1437297
St. Dev		482.386		605.975		598.306
% RSD		0.04		0.16		0.04

Table No. Robustness for Sodium Methyl Paraben, Change in Flow rate:

Statistics	Flow rate 1.1 ml/min		Flow rate 1.3 ml/min	
	RT	**Peak area**	**RT**	**Peak area**
	6.8	135053	5.8	111660
	6.8	135158	5.7	111014
	6.7	135419	5.7	113540
	6.7	135217	5.7	113641
	6.7	135669	5.7	113610
Average	6.7	135303	5.7	112693
St. Dev		244.097		1259.279
% RSD		0.18		1.12

Table No: Robustness for Sodium Methyl Paraben, Change in Wavelength

Statistics	Wavelength 213 nm		Wavelength 217	
	RT	**Peak area**	**RT**	**Peak area**
	5.7	131497	5.7	87360
	5.7	129977	5.7	88113
	5.7	132213	5.7	88504
	5.7	131326	5.7	88575
	5.7	131364	5.7	88683
Average	5.7	131275		88247
St. Dev		810.008		540.475
% RSD		0.62		0.61

Accuracy (Recovery):

Mean recovery for Methyl Paraben, Propyl Paraben, Benzyl Alcohol and Sodium Methyl Paraben from marketed formulations are listed in tables no.

% Recovery for MP

Concentration at specific level	Preservative added (mg/ml)	Peak area	Mean Recovered
50%	27.5	898102	27.81
100%	55	1790791	55.49
150%	82.5	2675328	82.94

% Recovery forPP

Concentration at specific level	Preservative added (mg/ml)	Peak area	Mean Recovered
50%	10	281410	9.86
100%	20	563526	19.74
150%	30	850822	29.74

Table No :% Recovery for BA

Concentration at specific level	Preservative added (mg)	Peak area	Mean Recovered
50%	50	1226548	50.19
100%	100	2464657	101.34
150%	150	3611203	148.48

Table No: % Recovery for Sodium Methyl Paraben

Concentration at specific level	Preservative added (mg/ml)	Peak area	Mean Recovered
50%	3.75	74707	3.7
100%	7.5	149926	7.5
150%	11.25	226185	11.2

Specificity

There was no interference of other excipients in peaks of preservatives.

Solution Stability

Table No: Solution Stability: Standard Solutions after 12 hours

Statistics	MP		PP		BA		SMP	
	RT	**Peak area**	**RT**	**Peak area**	**RT**	**Peak area**	**RT**	**Peak area**
2-8 “C	6.1	1964752	15.7	562293	4.9	2536147	6.2	124594
25” C	6.1	1963493	15.7	565473	4.9	2535221	6.4	124903
Average	6.1	1964123	15.7	563883	4.9	2535684	6.3	124749
St. Dev	0	890.25		2248.60		654.78		218.50
% RSD	0	0.05		0.40		0.03		0.18

Table No: SolutionStability: Standard Solutions after 24 hours

Statistics	MP		PP		BA		SMP	
	RT	**Peak area**	**RT**	**Peak area**	**RT**	**Peak area**	**RT**	**Peak area**
2-8 “C	6.1	1972976	15.7	579340	4.9	2543910	6.2	123182
25” C	6.1	1967869	15.7	582770	4.9	2562937	6.1	125420
Average	6.1	1970423	15.7	581055	4.9	2553424	6.2	124301
St. Dev		3611.19		2425.38		13454.12		1582.50
% RSD		0.18		0.42		0.53		1.27

Table No: Solution Stability: Sample Solutions (Fasimec Suspension)after 12 hours

Statistics	MP		PP		BA	
	RT	**Peak area**	**RT**	**Peak area**	**RT**	**Peak area**
2-8 “C	6.1	1719873	15.7	526864	4.9	2381283
25” C	6.1	1725304	15.7	526750	4.9	2390807
Average	6.1	1722589	15.7	526807	4.9	2386045
St. Dev		3840.30		80.61		6734.48
% RSD		0.22		0.02		0.28

Table No: Solution Stability: Sample Solutions (Fasimec Suspension) after 24 hours

Statistics	MP		PP		BA	
	RT	**Peak area**	**RT**	**Peak area**	**RT**	**Peak area**
2-8 “C	6.1	1735195	15.7	541991	4.9	2384737
25” C	6.1	1736138	15.7	544729	4.9	2396914
Average	6.1	1735667	15.8	543360	4.9	2390826
St. Dev		666.80		1936.06		8610.44
% RSD		0.04		0.36		0.36

Table No: Solution Stability: Sample Solutions included Sodium Methyl Paraben

Statistics	FASINEX SUSPENSION				ENDEX SUSPENSION			
	After 12 hours		After 24 hours		After 12 hours		After 24 hours	
	RT	**Peak area**	**RT**	**Peak area**	**RT**	**Peak area**	**RT**	**Peak area**
2-8 “C	6.1	126226	6.2	125224	6.1	123282	6.2	123248
25” C	6.4	126928	6.1	126261	6.4	124205	6.2	124521
Average	6.3	126577	6.2	126119	6.3	123744	6.2	123584
St. Dev		496.39		469.69		652.66		649.65
% RSD		0.39		0.32		0.53		0.52

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