Validation of Novel HPLC method for the estimation of selective drugs in bulk and pharmaceutical formulations

Anis Shaikh[1*], Priyanka Soni [2]

1. PhD. Research Scholar, Faculty of Pharmacy (B.R. Nahata College of Pharmacy), Mandsaur University, Mandsaur-458001
2. Faculty of Pharmacy (B.R. Nahata College of Pharmacy), Mandsaur University, Mandsaur-458001

Author for Correspondence: Anis Shaikh

anisshaikh63@gmail.com

ABSTRACT

AIM- To develop Validation of novel HPLC method for the estimation of selective drugs in bulk and pharmaceutical formulations. **MATERIAL & METHODS-** Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different concentrations and areas for each concentration were recorded three times, and mean area was calculated. Where to a reanalyzed sample solution, standard solutions of all the three drugs were added equivalent to 80, 100 and 120% of its drug content. Recovery study was carried by doing replicate study. The repeatability was performed for five replicates at five concentrations in linearity range for SOL and for MIB indicates the precision under the same operating condition over short interval time. Results of repeatability are reported in table respectively. Intermediate precision study was carried out by intra-day and inter-day precision study. Interday precision means precision study carried out on different days and intra day precision means precision study carried out at the same day on different time interval by the same solution. **RESULTS-** The system suitability parameter was carried out to verify that the analytical system was working properly and could give accurate and precise result. The six replicates of reference standard, 5µg/ml of SOL and MIB were injected separately and chromatogram was recorded. The linearity of analytical method was carried out to check its ability to elicit test results that are proportional to the concentration of analyte in sample within a given range. Different levels of standard solutions were prepared and injected into the HPLC and the chromatogram was recorded. The

recovery of added standards (80%, 100% and 120%) was found at three replicate and three concentrations level. The value of % means just close to 100, SD and % RSD are less than 2 indicate the accuracy of method. **CONCLUSION-** Quantitative analysis of multi-component formulations usually requires a prior extraction of drugs from the recipients of drugs or elaborate separation procedures for multiple drugs themselves.

KEYWORDS-

Validation, Novel HPLC method, Estimation of selective drugs, Bulk and pharmaceutical formulations, Intermediate precision

DOI: 10.48047/ecb/2022.11.12.43

INTRODUCTION

Today, chromatographic techniques have little to do with the separation of color (the technique names evolved from the earliest work of separating dyes or plant pigments on paper), but do involve the separation of compounds in a sample mixture. A number of types of separation methods have developed over the years to accommodate the various physical and chemical states of sample mixtures one may be interested in separating and analyzing [1-3]. The feature that distinguishes chromatography from most other physical and chemical methods of separation is that, two mutually immiscible phases brought into contact; one phase is stationary and other mobile [4]. The mobile phase can be gas or a liquid, where as the stationary phase can only be a liquid or a solid. When the separation involves predominantly a simple portioning between two immiscible liquid phases, one stationary and other mobile, the process is called liquid-liquid chromatography. When physical surface forces are mainly involved in the retentive ability of the stationary phase, the process is denoted as liquid solid chromatography. Liquid chromatography has been performed in a column or on an open bed [5-7]. Chromatography is probably the most power full and versatile analytical technique available to modern chemist. Its power arises from its capacity to determine quantitatively many individual components present in the mixture in one, single analytical procedure. Its versatility comes from its capacity to handle a very wide

verity of samples that may be liquid, gaseous or solid in nature. In addition the sample can range in complexity from the single substance to multicomponent mixture containing widely differing chemical species [8-10].

Although literature survey reveals that there were both spectroscopic and chromatographic methods reported, both for single estimation and in combination with others drugs for both the two drugs. But no spectroscopic and chromatographic method has been reported for the analysis of these drugs in combination. The non-availability of spectroscopic and chromatographic method until now for the simultaneous estimation of this combination made it a worthwhile objective to pursue the present research work. It has been also planned to validate the developed methods as per ICH norms. Therefore, in the proposed project work, a successful attempt has been made to developed simple, precise and accurate methods for the simultaneous analysis of Solifenacin and Mirabegron in tablet dosage form.

MATERIAL AND METHOD

Validation of Developed Method

A. Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different concentrations and areas for each concentration were recorded three times, and mean area was calculated. The regression equation and correlation coefficient of curve are given and the standard calibration curve of the drug is shown in figure. From the mean of AUC observed and respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration [11].

B. Accuracy (Recovery study)

To check the accuracy of the developed method recovery study was carried out as per ICH norms. Where to a reanalyzed sample solution, standard solutions of all the three drugs were added equivalent to 80, 100 and 120% of its drug content. Recovery study was carried by doing replicate study. The result and its statistical result are reported in Table respectively [12].

C. Precision Study

1. Repeatability

The repeatability was performed for five replicates at five concentrations in linearity range for SOL and for MIB indicates the precision under the same operating condition over short interval time. Results of repeatability are reported in table respectively [13].

2. Intermediate precision

Intermediate precision study was carried out by intra-day and inter-day precision study. Interday precision means precision study carried out on different days and intra day precision means precision study carried out at the same day on different time interval by the same solution. Here six replicates of sample solutions were prepared from the stock solution. For intra-day precision study concentration of all the three drugs were calculated for three times on the same day at an interval of 1hr. The results of the intra-day precision along with their statistical validation are reported in Table 5.18. In inter-day study, the concentration of drug contents was calculated on three different days 1st, 2nd and 3rd day. The results of the inter day precision along with their statistical validation are reported are given in Table [14].

D. LOD and LOQ study

LOD and LOQ values were calculated to check the detection limit and quantitation limit of the method by using following equations.

$$LOD = \frac{3.3\sigma}{S}$$

$$LOQ = \frac{10\sigma}{S}$$

Where, σ is the standard deviation and

S is the slope of the curve.

E. Selectivity and specificity

Selectivity of the method towards the drugs was established through study of resolution factor between the drug peaks. Under the proposed chromatographic conditions both the drugs were completely separated from each other with a resolution of 2.05 between SOL and MIB. It indicates that the method is selective for simultaneous estimation. Specificity was assessed by comparing the

chromatograms of tablet solution with the placebo solution and also with the chromatograms obtained from standard drugs. As the retention time for both the drugs were same in standard solution as well as in tablet solution and also there was no extra peak co-eluted for diluents indicated the specificity of the method for quantitative estimation of these drugs in commercial formulation [15-17].

F. Robustness

As per ICH norms, small, but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The study wasperformed by change in ratio of mobile phase.

System-suitability conditions

Under the optimum chromatographic conditions, the retention times obtained for SOL and MIB were 4.918 and 3.256 min respectively. The resolution (Rs) between ATOR and PIO was 2.05. The result of capacity factor, tailing factor, theoretical plate number are reported in Table 5.23. The values obtained for these properties shows ($1 < k < 10$, R_S, > 2) this chromatographic conditions are appropriate for separation and quantification of all the three compounds. The number of plates (N) is a measure of column efficiency; which shows the good separation efficiency of the column used [18].

RESULTS & DISCUSSION

LINEARITY

Table 1: Linearity of SOL

Conc.	**Peak Area**						**Mean ± SD**
	Replica 1	**Replica 2**	**Replica 3**	**Replica 4**	**Replica 5**	**Replica 6**	
5	1006076	1017095	999913	1010687	1024862	1031318	1014992 ± 11779.49
10	2064509	2055823	2058860	2049876	2078374	2066234	2062279 ± 9867.805
15	3030965	3031193	3036464	3028496	3026468	3048034	3033603 ± 7825.547
20	4011512	4004965	4012549	4038380	4009315	4043755	4020079 ± 16552.16
25	5102162	5164099	5187894	5156518	5171163	5161573	5157235 ± 29097.92

Table 2: Response Ratio Data for Linearity of SOL

Replicates	Concentration (µg/ml)	Mean AUC	Response Ratio
Rep-1	5	1014992	202998.4
Rep-2	10	2062279	206227.9
Rep-3	15	3033603	202240.2
Rep-4	20	4020079	201004
Rep-5	25	5157235	206289.4
Mean		203752	
SD			50.11
%RSD			0.65

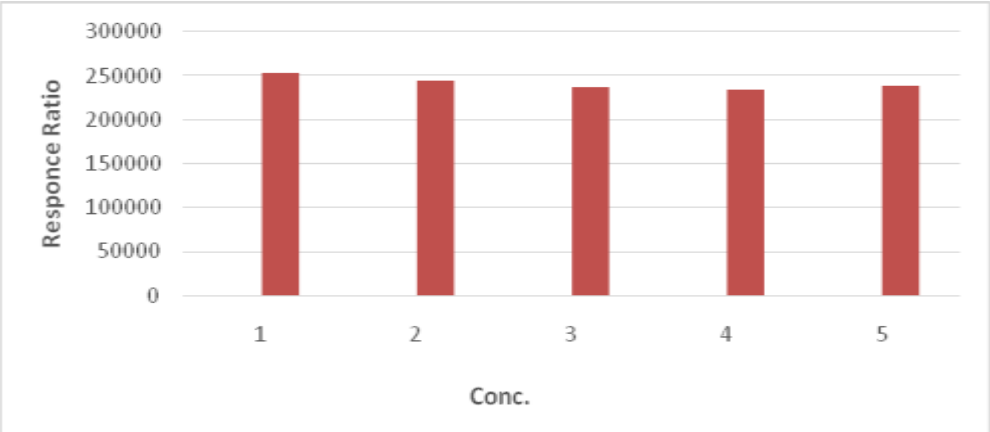

Fig 1: Response Ratio Curve of SOL

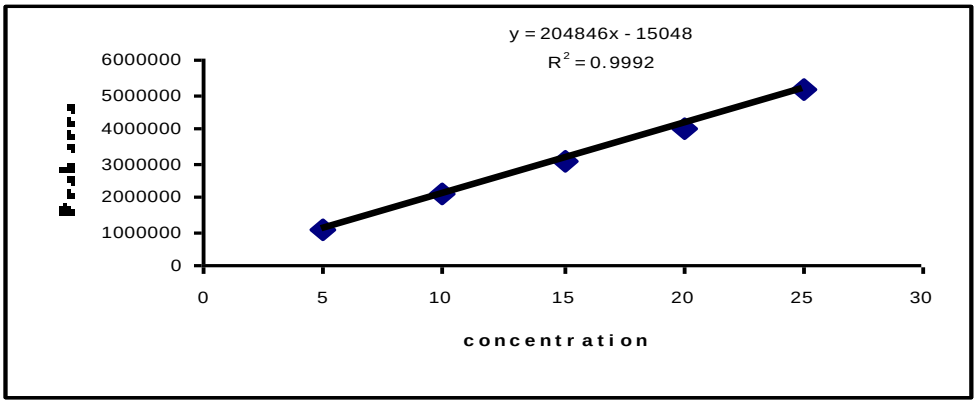

Fig 2: Calibration curve of SOL

Mean standard deviation	20153.02
Slope	204846
LOD	3.3 x 20153.02/204846= 0.28 μg/ml
LOQ	10 x 20153.02/204846= 0.86 μg/ml

Table 3: Linearity of MIB

Conc.	**Peak Area**						**Mean ± SD**
	Replica 1	**Replica 2**	**Replica 3**	**Replica 4**	**Replica 5**	**Replica 6**	
5	1278319	1251698	1261677	1256021	1286251	1277015	1268497 ± 3918.35
10	2430400	2456558	2423352	2472280	2406928	2445956	2439246±23712.02
15	3532244	3531129	3591347	3572418	3519401	3548518	3549176±27633.15
20	4696147	4681402	4693653	4684429	4660450	4666927	4680501±14285.02
25	5953012	5938715	5973641	5992403	5988563	5981579	5971319±21216.58

Table 4: Response Ratio Data for Linearity of MIB

Replicates	**Concentration (μg/ml)**	**Mean AUC**	**Response Ratio**
Rep-1	5	1268497	253699.4
Rep-2	10	2439246	243924.6
Rep-3	15	3549176	236611.7
Rep-4	20	4680501	234025.1
Rep-5	25	5971319	238852.8
Mean			241422.7
SD			66.95
%RSD			0.87

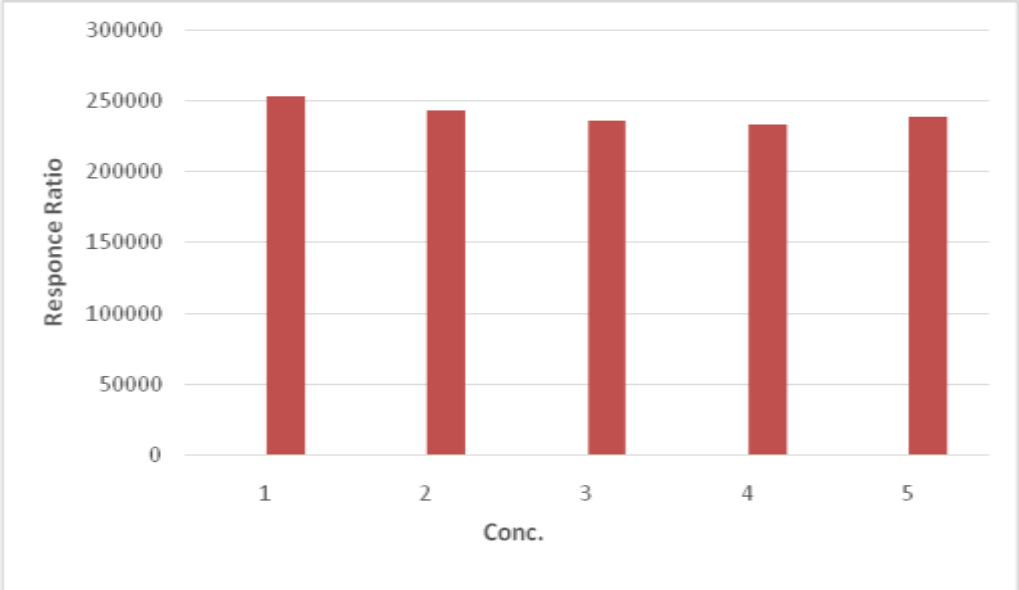

Fig. 3: Response Ratio Curve of MIB

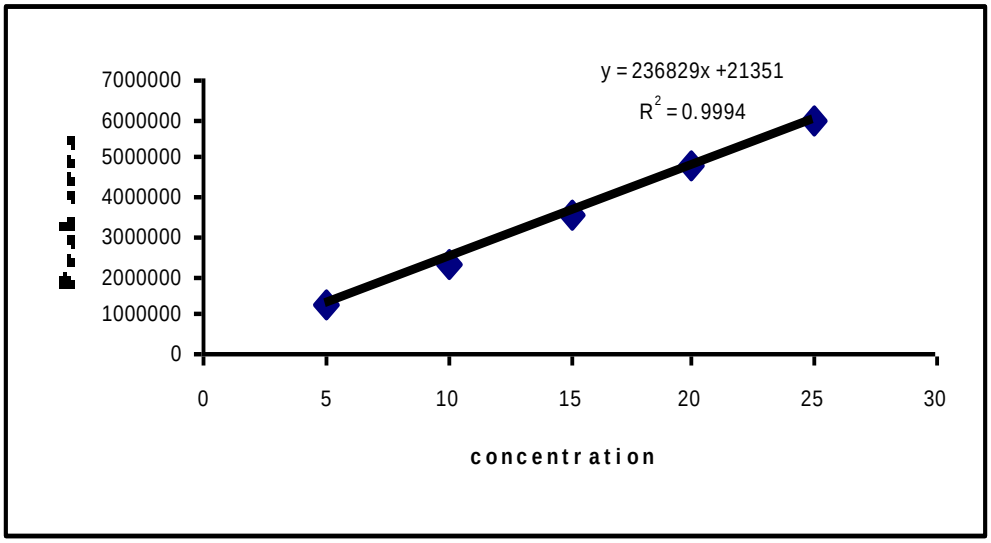

Fig 4: Calibration curve of MIB

Mean standard deviation	19596.7
Slope	236829
LOD	3.3 x 19596.7/236829= 0.27 µg/ml
LOQ	10 x 19596.7/236829= 0.82 µg/ml

ACCURACY

Table 5: Result of recovery study

S. No.	Label Claim (mg/tablet)		Amount added			% Recovery	
	SOL	RAB	%	SOL	RAB	SOL	RAB

Replicate 1	500	500	80	400	400	99.64	99.89
Replicate 2	500	500		400	400	98.86	98.98
Replicate 3	500	500		400	400	98.84	99.80
Replicate 1	500	500	100	500	500	99.73	99.87
Replicate 2	500	500		500	500	99.38	99.25
Replicate 3	500	500		500	500	98.66	98.16
Replicate 1	500	500	120	600	600	98.54	98.67
Replicate 2	500	500		600	600	98.49	98.98
Replicate 3	500	500		600	600	99.38	99.63

Table 6: Result of statistical validation of recovery study

%	**Drug**	**Mean %± S.D.**	**% CV**	**S.E.**
80	**SOL**	99.11 ± 0.358	0.35	0.32
	MIB	99.37 ±0.371	0.37	0.31
100	**SOL**	99.26 ± 0.489	0.48	0.47
	MIB	99.37 ±0.501	0.37	0.37
120	**SOL**	98.80 ± 0.553	0.55	0.29
	MIB	98.42 ±0.587	0.60	0.33

PRECISION STUDY

Repeatability

Table 7: Repeatability of SOL

CONC. / **REP.**	**CONCENTRATION FOUND (µg/ml)**					**MEAN**
	5	10	15	20	25	
Replicate-1	4.96	9.95	14.98	19.95	24.98	
Replicate-2	4.12	9.99	14.95	19.94	24.95	
Replicate-3	4.98	9.05	14.08	19.05	25.05	
Replicate-4	4.99	10.95	14.01	19.03	24.95	
Replicate-5	4.08	10.99	14.04	19.98	25.02	
MEAN	4.317	10.088	14.810	19.91	24.99	

% MEAN	99.16	100.417	99.333	99.792	99.833	99.908
SD	0.070	0.041	0.051	0.048	0.044	0.051
% RSD	6.838	2.061	1.684	1.214	0.885	2.536

Table 8: Repeatability of MIB

CONC. / **REP.**	**CONCENTRATION FOUND (µg/ml)**					**MEAN**
	5	10	15	20	25	
Replicate-1	4.86	9.85	14.08	19.85	24.78	
Replicate-2	4.24	9.80	14.78	19.84	24.01	
Replicate-3	4.81	9.15	14.21	19.11	25.00	
Replicate-4	4.84	10.10	14.25	19.05	24.85	
Replicate-5	4.10	10.65	14.01	19.99	25.01	
MEAN	4.417	9.58	14.510	19.72	24.85	
% MEAN	99.792	99.958	99.736	99.979	99.967	99.886
SD	0.041	0.037	0.068	0.039	0.042	0.045
% RSD	1.018	0.465	0.571	0.245	0.210	0.502

Intermediate precision

Table 9: Result and statistical validation of intraday precision study

Replicate No.	**Concentration found (µg/ml)**					
	1st h.		**2nd h.**		**3rd h.**	
	SOL	**MIB**	**SOL**	**MIB**	**SOL**	**MIB**
1	10.11	30.32	10.16	30.03	10.30	30.12
2	10.12	30.09	10.03	30.23	10.29	30.11
3	10.10	30.11	10.11	30.04	10.19	30.17
4	10.31	30.21	10.21	30.10	10.22	30.19
5	10.21	30.18	10.23	30.21	10.31	30.22
6	10.09	30.19	10.09	30.11	10.23	30.21
Mean	10.15	30.18	10.13	30.12	10.27	30.17
± S.D.	0.086	0.819	0.076	0.083	0.049	0.040

% CV	0.85	0.27	0.75	0.28	0.48	0.15
S.E.	0.0353	0.0334	0.0310	0.0343	0.0203	0.0188

Table 10: Result and statistical validation of inter-day precision study

Replicate No.	**Concentration found (μg/ml)**					
	1st day.		**2nd day.**		**3rd day.**	
	SOL	**MIB**	**SOL**	**MIB**	**SOL**	**MIB**
1	10.21	30.19	10.01	30.12	10.31	30.03
2	10.09	30.21	10.19	30.23	10.21	30.23
3	10.21	30.12	10.18	30.08	10.22	30.12
4	10.11	30.13	10.21	30.12	10.32	30.21
5	10.14	30.09	10.20	30.21	10.11	30.33
6	10.21	30.11	10.03	30.11	10.21	30.32
Mean	10.16	30.14	10.13	30.14	10.23	30.20
± S.D.	0.055	0.047	0.091	0.060	0.077	0.047
% CV	0.54	0.16	0.90	0.20	0.75	0.38
S.E.	0.022	0.019	0.037	0.024	0.031	0.047

LOD and LOQ OF SOL and MIB

Table 11: LOD and LOQ OF SOL and MIB

Name	**LOD (μg/ml)**	**LOQ (μg/ml)**
SOL	0.28	0.86
MIB	0.27	0.82

SELECTIVITY AND SPECIFICITY

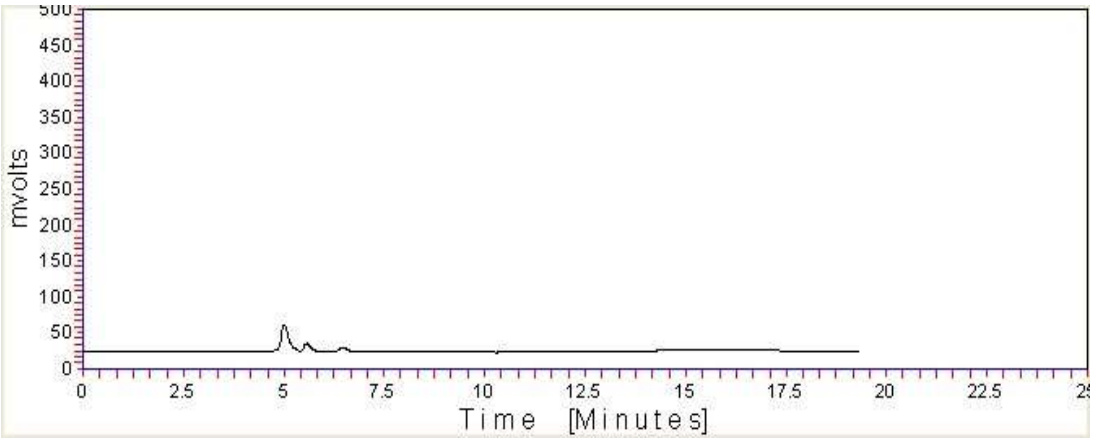

Fig.5: Chromatogram of blank

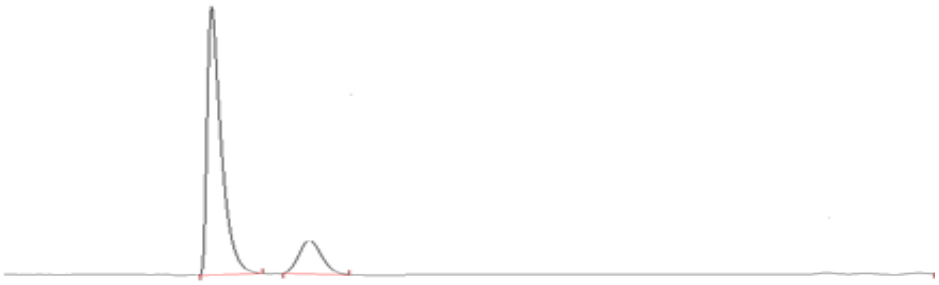

Fig.6: Chromatogram of both drugs

ROBUSTNESS

Table 12: Robustness of SOL

CONC. / REP.	Concentration Found (µg/ml)					MEAN
	5	10	15	20	25	
Replicate-1	4.86	9.85	14.08	19.85	24.78	
Replicate-2	4.24	9.80	14.78	19.84	24.01	
Replicate-3	4.81	9.15	14.21	19.11	25.00	
Replicate-4	4.84	10.10	14.25	19.05	24.85	
Replicate-5	4.10	10.65	14.01	19.99	25.01	
MEAN	4.417	9.58	14.510	19.72	24.85	
% MEAN	99.792	99.958	99.736	99.979	99.967	99.886
SD	0.041	0.037	0.068	0.039	0.042	0.045
% RSD	1.018	0.465	0.571	0.245	0.210	0.502

Table 13: Robustness of MIB

CONC. / REP.	Concentration Found (μg/ml)					MEAN
	5	10	15	20	25	
Replicate-1	4.96	9.95	14.98	19.95	24.98	
Replicate-2	4.12	9.99	14.95	19.94	24.95	
Replicate-3	4.98	9.05	14.08	19.05	25.05	
Replicate-4	4.99	10.95	14.01	19.03	24.95	
Replicate-5	4.08	10.99	14.04	19.98	25.02	
MEAN	4.317	10.088	14.810	19.91	24.99	
% MEAN	99.16	100.417	99.333	99.792	99.833	99.908
SD	0.070	0.041	0.051	0.048	0.044	0.051
% RSD	6.838	2.061	1.684	1.214	0.885	2.536

SYSTEM-SUITABILITY CONDITIONS

Table 14: Result from system suitability study

Property (n=6)	SOL	MIB
Rt	4.918	3.256
T_f	1.37	1.22
K	1.23	1.51
N	8992	7696
Rs	-	2.05

Rt: Retention time, T_f : Tailing factor, k7: Capacity factor, N : Theoretical plates number Rs: Resolution.

The RP-HPLC method was developed for estimation of Mirabegron (MIB) and Solifenacin (SOL) incombined formulation by isocratically using Methanol: Acetonitrile: Water in 55:30:10 (v/v) as mobile phase. Luna C_{18} (μmx25cmx4.6mm i.d.)column as stationary phase and chromatogram was recorded at 239nm. Then developed method was validated by using various parameters. The system suitability parameter was carried out to verify that the analytical system was working properly and could give accurate and precise result. The six replicates of reference standard, 5μg/ml ofSOL and MIB were injected separately and chromatogram was recorded

[19]. The linearity of analytical method was carried out to check its ability to elicit test results that are proportional to the concentration of analyte in sample within a given range. Different levels of standard solutions were prepared and injected into the HPLC and the chromatogram was recorded. The results of linearity are reported in table. Specificity of the method was determined and the peaks of diluent, mobile phase and excipient of physical mixture did not interfere with standard peaks of SOL and MIB. The validity and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100% and 120%) was found at three replicate and three concentrations level. The value of % means just close to 100, SD and % RSD are less than 2 indicate the accuracy of method. Result of recovery study shown in table. Precision was determined by repeatability and Intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The robustness of developed method was checked by changing in the deliberate variation in solvent [20].

CONCLUSION

Quantitative analysis of multi-component formulations usually requires a prior extraction of drugs from the recipients of drugs or elaborate separation procedures for multiple drugs themselves. Regardless of the various pharmaceutical and reported methods available, development of a simple and systematic procedure that given as clear separation of drugs is a difficult endeavor. The presence of additives and decomposition products further complicates the development of analytical procedures.

REFERENCES

1. Billiet, H.A.H., and Rippel, G., Method Development and Selectivity Optimization in High-Performance Liquid Chromatography; In Advances in Chromatography, Marcel Dekker, Inc, New York, 1998, Vol. 39, 263-310.
2. Sharaf, M.A., Assessment of Chromatographic Peak Purity; In Advances in Chromatography, Market Dekker, Inc, New York, 1997, Vol- 37, 1-6.
3. Sadek, P.C., Troubleshootings HPLC Systems;In A Bench manual, John Wiley and Sons, Linc, New York, 2000,109-190.

4. Troubleshootings, Cause and solution, www.hplcsystems.com.
5. A fitness for Purpose of Analytical method; a laboratory Guide to method validation and Related Topics, Eurachem, 1998.
6. Code Q2A-Text on validation of analytical procedure Step-3 Consensus Guideline, 1994, ICH Harmonised Tripartite Guideline.
7. Code Q2B- validation of analytical procedure Methodology Step-4 Consensus Guideline, 1994, ICH Harmonised Tripartite Guideline.
8. Validation of Analytical Procedure- Definition and Terminology, FDA Center for Veterinary Medicine Guidance Document. 63, 1999.
9. Guideline for industry, Analytical Procedure and method validation, FDA, 49Aug, 2000.
10. Singh, S. and Garg. S., Understanding; Analytical Method Validation, Pharma Times, Aug, 1999, 15-20.
11. Huber, L., Validation of Analytical Methods; Review and Strategy, LC-GC International Feb, 1998, 99-105.
12. Munson JW. (2001). *Pharmaceutical Analysis- Modern Methods.* (Part-B), Marcel Dekker publisher's, pp 16-18
13. Sharma BK. (2005). *Instrumental methods of chemical analysis.* Goel Publishing house, Meerut, ($24^{th}ed^{n}$), pp C-286-C-311
14. Gopinath R, Rajan S, Meyyanathan SN, Krishnaveni N, Suresh BA. (2007). RP-HPLC method for simultaneous estimation of Solifenacin and aceclofenac in tablets. *Indian Journal of Pharmaceutical Science,* 69(2):137-140
15. Mahaparale PR, Sangshetti JN, Kuchekar BS. (2007). Simultaneous Spectrophotometric estimation of aceclofenac and Solifenacin in tablet dosage form. *Indian Journal of Pharmaceutical Science*, 69(3):289-292
16. Patel PM, Desai HJ, Patel RC, Patel NM. (2007). Spectrophotometric method for estimation of rabeprazole. *Indian Journal of Pharmaceutical Science*, 69(3):318-320
17. Prasad CVN, Parihar C, Sunil P, Parimoo P. (1997). Simultaneous determination of amlodipine HCL, Hydrochlorthiazide and atenolol in combined formulation derivative spectroscopy. *Journal of Pharmaceutical and Biomedical Analysis*, 39: 877-884.

18. Sankar PR, Kishore KP, Babji B and SulthanaMS: Analytical method development and validation for the determination ofmirabegron in pharmaceutical dosage formby RP-HPLC. Int J Pharm Sci & Res 2020;11(5): 2223-28.
19. Jadhav R.A, Sanil Y.M., Shankarwar, S.G. etal. Development and Validation of Rapid Stability-Indicating RP-HPLC Method forAssay and Related Substances of Solifenacin Succinate. Chromatographia 83,(2020) 1107–1119.
20. Van Teijlingen R, Meijer J, TakusagawaS,vanGelderen M, van den Beld C, Usui T.Development and validation of LC-MS/MSmethods for the determination ofmirabegron and its metabolites in humanplasma and their application to a clinicalpharmacokinetic study. J Chromatogr B AnalTechnol Biomed Life Sci. (2012) 887–888:102–11.