STABILITY INDICATING HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATIONS OF ATENOLOL AND NIFEDIPINE IN BULK AND TABLET DOSAGE FORM

[1]Ansari Yaasir Ahmed*, [2]Dr. Sumer Singh, [3]Dr. Majaz Quazi, [1]Jameel Ahemad, [1]Ansari Mohd. Razi
[1]Research Scholar, School of pharmacy and medical sciences, Singhania University, Pacheri Bari, Dist-Jhunjhunu
[2]Asso. Professor, School of pharmacy and medical sciences, Singhania University, Pacheri Bari, Dist-Jhunjhunu
[3]Asso. Professor, Ali Allana College of Pharmacy, Akkalkuwa, North Maharashtra University, Jalgaon.
[1]Department of Pharmacy and Medical Sciences,
[1]Singhania University, Pacheri Bari, Dist-Jhunjhunu, India.

A new HPLC method has been developed and validated with different parameters for Atenolol and Nifedipine in combine dosage form. The chromatograms were developed using a mobile phase of MeOH: OPA (70:30) with a flow rate of 0.7 ml/min. C18 Column of 4.6 x 250 mm dimension was used as a stationary phase, particle size 5µm. The detection was carried out at 233 nm. The method was validated according to ICH guidelines for linearity, precision, Repeatability, LOD and LOQ. The response was found to be linear in concentration range of 1-5 mcg/ml for Nifedipine and 20-100 mcg/ml for Atenolol. The LOD and LOQ were found to be 0.1415 and 0.4289 respectively for Atenolol and 0.1834 and 0.5558 respectively for Nifedipine. The developed method was simple, precise, accurate and reproducible and therefore suitable for routine analysis of drugs in tablet dosage form. The stability study also done through exposure of analyte solution to five different stress conditions.

Keywords: HPLC, Atenolol, Nifedipine, Development, Validation.

1. INTRODUCTION

Atenolol and Nifedipine are Anti-hypertensive drugs. Nifedipine is a drug used to manage Angina, high blood pressure, Reynaud's phenomenon and premature Labour. It is one of the choices of drug for Prinzmetal Angina. It May be used to treat high blood pressure in pregnancy. Atenolol is a selective β_1 receptor antagonist, a drug belonging to the group of beta blockers , a class of drugs used primarily in cardiovascular diseases. Introduced in 1976, Atenolol was developed as a replacement for propranolol in the treatment of hypertension. It works by slowing down the heart and reducing its workload.

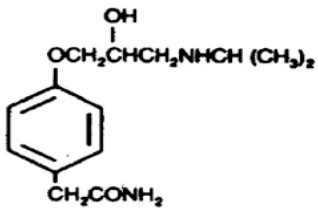

Fig.1.1: Structure of Atenolol

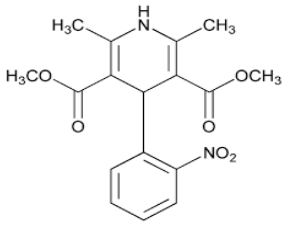

Fig.1.2: Structure of Nifedipine

High Performance Liquid Chromatography: (HPLC)

HPLC is one type of Chromatography to separate Ionic species and macromolecules. Chromatographic separation in HPLC is a result of specific interaction of drug with mobile and stationary phase. Mobile phase run the solution of drug through the column. Column acts as stationary phase. HPLC contains different parts from Mobile phase reservoir, Degasser, column to the detector for analysing different samples.

HPLC Method development involves the determination of Theoretical plate, Tailing factor and Resolution. Method validation as per ICH involves parameters are Linearity, Accuracy, Repeatability, Robustness, LOD and LOQ etc, by which developed method is validated.

2. MATERIAL AND METHOD

Chromatographic conditions:

The following chromatographic conditions were established by trial and error and were kept constant throughout the experimentation-

Table No-2.1 Chromatographic conditions:

HPLC	AGILENT (1100) Gradient System UV detector
Software	Chemstation
Column	id 4.6 x 250 mm length
Particle size packing	5 µm
Stationary phase	C18 (AGILENT)
Mobile Phase	Methanol: 0.05 % OPA (70:30)
Detection Wavelength	233 nm
Flow rate	0.7 ml/min
Temperature	Ambient
Sample size	20 µl

Reagents and Chemicals:

ATE and NIFE reference standards were supplied by J.B Chemicals, Ankleshwar, India. Pharmaceutical dosage form (Beta-Nifedine Tablet) containing ATE and NIFE was obtained commercially. This tablet contained ATE 50 mg and NIFE 20 mg. Methyl alcohol and O-Phosphoric as HPLC grade were used as solvents.

Standard stock solution of ATE and NIFE (Mixed):

Accurately weigh 50 mg ATE and 20 mg NIFE. Dissolve in methanol and make volume upto 10ml. standard solution contains 5000 µgm/ml of ATE and 2000 µgm/ml NIFE. (Stock solution I)

1) Take 0.05 ml from stock solution and make up vol. 10 ml with M.P = 25 µg/ml ATE & 10 µg/ml NEFI
2) Take 0.1 ml from stock and make up vol. 10 ml with MP = 50 µg/ml ATE & 20 µg/ml NEFI
3) Take 0.15 ml from stock and make up vol. 10 ml with MP = 75 µg/ml ATE & 30 µg/ml NEFI
4) Take 0.2 ml from stock and make up vol. 10 ml with MP = 100 µg/ml ATE & 40 µg/ml NEFI
5) Take 0.3 ml from stock and make up vol. 10 ml with MP = 150 µg/ml ATE & 60 µg/ml NEFI

Tablet solution Preparation for Assay:

Tablet (Beta-Nifidine) contains 50 mg ATE and 20 mg NIFE. 20 Tab. has been taken, weight of 20 tab. was 2.521 gm. Average weight of drug was 126 mg. Dissolve 126 mg of Tablet powder in 10 ml Vol. Flask and make volume upto the mark. It contains 2000 µgm/ml Atenolol & 5000 µg/ml Nifedipine (Stock Solution II). Take 0.3 ml from Stock Solution-II and make volume upto 10 ml with M.P. Now, it is 30 µgm/ml NIFE 75 µgm/ml ATE. This concentration is used for performing the assay.

3. Results and Discussion

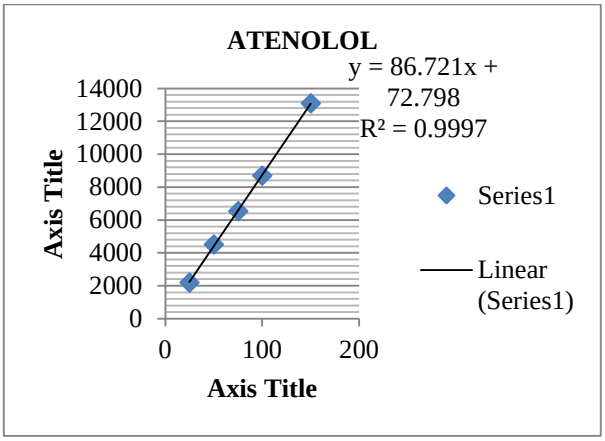

Fig.3.1 Linearity Study of ATE

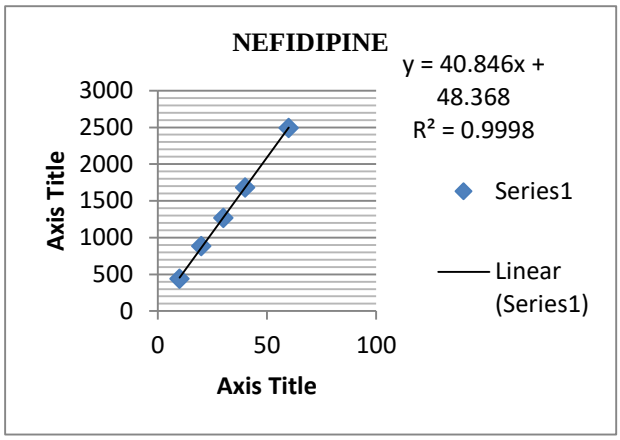

Fig.3.2 Linearity Study of NIFE

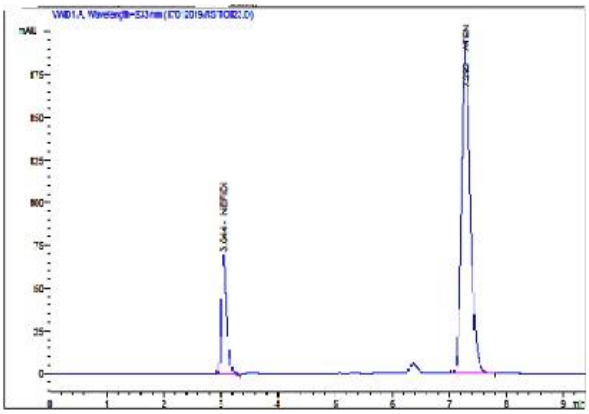

Fig.3.3 Precision of ATE and NIFE

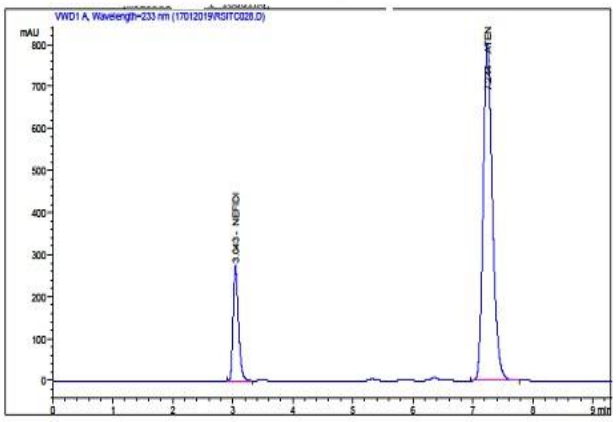

Fig.3.4 System Suitability Test of ATE and NIFE

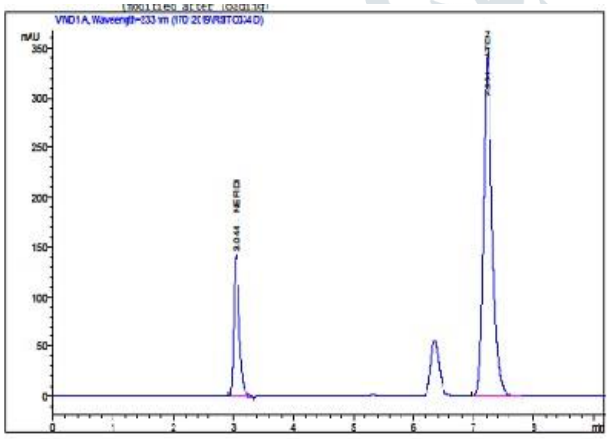

Fig.3.5 % Recovery of ATE and NIFE

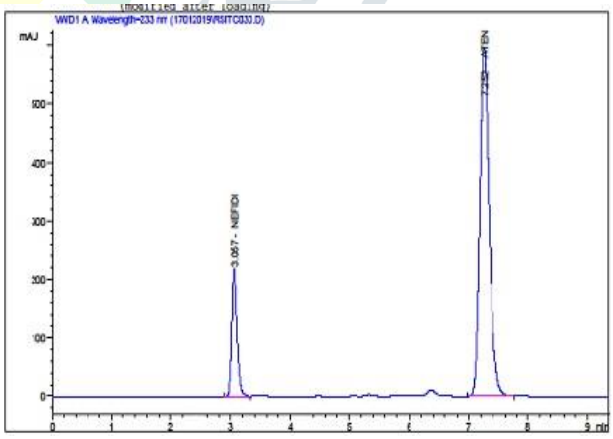

Fig.3.6 % Recovery of ATE and NIFE

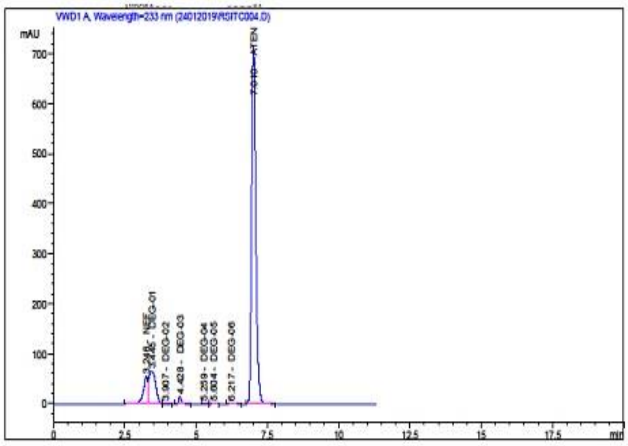

Fig.3.7 Acid Degradation

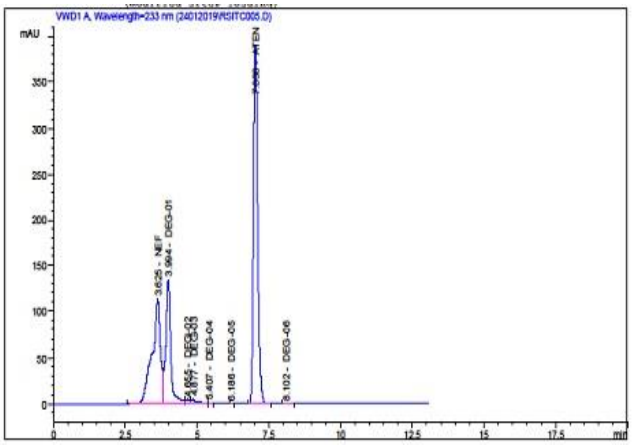

Fig. 3.8 Alkaline Degradation

Table No: 3.1 Linearity

Atenolol								
Sr No.	Conc.	Area I	Area II	Mean	SD	%RSD	Sr No.	Conc.
1	25	2200.01	2196.65	2198.33	2.38	0.11	1	25
2	50	4516.91	4514.09	4515.5	1.99	0.04	2	50
3	75	6552.25	6525.06	6538.66	19.23	0.29	3	75
4	100	8710.77	8679.58	8695.18	22.05	0.25	4	100
5	150	13108.5	13101	13104.75	5.30	0.04	5	150
Nifedipine								
1	10	441.63	442.82	442.23	0.84	0.19	1	10
2	20	884.54	883.85	884.20	0.49	0.06	2	20
3	30	1272.91	1268.78	1270.85	2.92	0.23	3	30
4	40	1687.37	1683.12	1685.25	3.01	0.18	4	40
5	60	2494.52	2494.83	2494.68	0.22	0.01	5	60

Table No-3.1 displayed the linearity study of ATE and NIFE. ATE used in a concentration range of 25 to 150 µgm/ml. The mean areas of different concentration obtained were 2198.33, 4515.5, 6538.66, 8695.18, and 13104.75. The %RSD for these concentrations was 0.11, 0.04, 0.29, 0.25, and 0.04 respectively. NIFE used in a concentration range of 10 to 60 µgm/ml. The mean areas of different concentration obtained were 442.23, 884.20, 1270.85, 1685.25 and 2494.68. The %RSD for these concentrations was 0.19, 0.06, 0.23, 0.18, and 0.01 respectively.

Table No: 3.2 Intraday Precision

Atenolol								
Sr No.	Conc	Area	II	Mean	Amt Found	% Amt Found	SD	%RSD
1	25	2219.41	2217.97	2218.69	24.74	98.98	1.02	0.05
2	75	6496.67	6447.95	6472.31	73.79	98.38	34.45	0.53
3	150	13057	13068.8	13062.90	149.79	99.86	8.34	0.06
Nifedipine								
1	10	442.4	452.58	447.49	9.77	97.73	0.96	0.89
2	30	1272.46	1271.18	1271.82	29.95	99.85	0.91	0.07
3	60	2491.98	2488.5	2490.24	59.79	99.65	2.46	0.10

Table No-3.2 displayed the study of Intraday Precision of ATE and NIFE. For this, ATE used in a concentration of 25 µgm/ml 75 µgm/ml and 150 µgm/ml. The % of amount found for these concentrations were 98.98, 98.38 and 99.86 respectively. The %RSD for these concentrations was 0.05, 0.53, and 0.06. NIFE used in a concentration of 10 µgm/ml 30 µgm/ml and 60 µgm/ml. The % of amount found for these concentrations were 97.73, 99.85 and 99.65 respectively. The %RSD for these concentrations was 0.89, 0.07, and 0.10.

Table No: 3.3 Interday Precision

Atenolol								
Sr No.	Conc.	Area I	Area II	Mean	Amt Found	% Amt Found	SD	%RSD
1	25	2222.31	2219.87	2221.09	24.77	99.09	1.73	0.08
2	75	6498.98	6456.98	6477.98	73.18	97.57	29.70	0.46
3	150	13057	13060.51	13058.76	149.74	99.83	2.48	0.02
Nifedipine								
1	4	441.29	455.54	448.42	9.76	97.95	0.96	0.21
2	12	1270.48	1274.98	1272.73	29.97	99.93	3.18	0.25
3	20	2492.54	2490.58	2491.56	59.82	99.70	1.39	0.06

Table No-3.3 displayed the study of Interday Precision of ATE and NIFE. For this, ATE used in a concentration of 25 µgm/ml 75 µgm/ml and 150 µgm/ml. The % of amount found for these concentrations were 99.09, 97.57 and 99.83 respectively. The %RSD for these concentrations was 0.08, 0.46, and 0.02. NIFE used in a concentration of 4 µgm/ml 12 µgm/ml and 20 µgm/ml. The % of amount found for these concentrations were 97.95, 99.93 and 99.70 respectively. The %RSD for these concentrations was 0.21, 0.25, and 0.06.

Table No: 3.4 Accuracy

Atenolol									
Sample Conc.	µgm/ml	Amt added	Area	Amt found	Amt rcvd	% rcvd	Mean	SD	%RSD
80%	25	20	3974.44	44.99	19.99	99.95	99.75	0.28	0.28
	25	20	3968.14	44.91	19.91	99.55			
100%	25	25	4406.37	49.97	24.97	99.88	99.70	0.25	0.26
	25	25	4398.86	49.88	24.88	99.52			
120%	25	30	4833.22	54.89	29.89	99.64	99.63	0.01	0.01
	25	30	4832.69	54.88	29.88	99.62			
Nifedipine									
80%	10	8	782.9	17.98	7.98	99.75	99.81	0.08	0.09
	10	8	783.11	17.99	7.99	99.87			
100%	10	10	864.26	19.97	9.97	99.70	99.50	0.28	0.28
	10	10	862.52	19.93	9.93	99.30			
120%	10	12	950.94	22.10	12.10	100.83	100.55	0.40	0.40
	10	12	948.13	22.03	12.03	100.26			

Table No-3.4 displayed the Accuracy (% Recovery) study of ATE and NIFE. For this, ATE used in a concentration of 80%, 100% and 120%. The mean % of amount recovered of these concentrations was 99.75, 99.70 and 99.63 respectively. NIFE also used in a concentration of 80%, 100% and 120%. The mean % of amount recovered of these concentrations was 99.81, 99.50 and 100.55 respectively.

Table No-3.4 Robustness (Atenolol)

Conc.(µgm/ml)	Area	Mean	SD	%RSD
Change in Flow rate (0.6 ml/min)				
25	2329.42	2322.27	10.12	0.44
25	2315.11			
Change in Flow rate (0.8 ml/min)				
25	1723.44	1721.96	2.09	0.12
25	1720.48			
Change in Mobile Phase Concentration (69:31)				
25	1962.81	1962.5	0.45	0.02
25	1962.18			
Change in Mobile Phase Concentration (71:29)				
25	1962.99	1963.43	0.62	0.03
25	1963.87			
Change in Wavelength (232 nm)				
25	1945.27	1942.4	4.07	0.21
25	1939.52			
Change in Wavelength (234 nm)				
25	1986.33	1984.58	2.47	0.12
25	1982.83			

Table No-3.4 displayed the Robustness study of ATE. Robustness studies of System were performed by changing the flow rate, M.P concentration and wavelength. The mean found were 2322.27 and 1721.96 for the flow rate of 0.6 ml/min and 0.8 ml/min respectively. The %RSD found were 0.44 and 0.12 for the flow rate of 0.6 ml/min and 0.8 ml/min respectively. The mean found were 1962.5 and 1963.43 for Mobile Phase Concentration (69:31) and Mobile Phase Concentration (71:29) respectively. The %RSD found were 0.02 and 0.03 for Mobile Phase Concentration (69:31) and Mobile Phase Concentration (71:29) respectively. The mean found were 1942.4 and 1984.58 for Wavelength (232 nm) and Wavelength (234 nm) respectively. The %RSD found were 0.21 and 0.12 for Wavelength (232 nm) and Wavelength (234 nm) respectively. This study shows that system is robust and withstand by changing different aspects of system.

Table No-3.5 Robustness (Nifedipine)

Conc.(µgm/ml)	Area	Mean	SD	%RSD
Change in Flow rate (0.6 ml/min)				
10	515.22	514.55	0.67	0.13
10	513.88			
Change in Flow rate (0.8 ml/min)				
10	385.29	385.93	0.91	0.23
10	386.57			
Change in Mobile Phase Concentration (69:31)				
10	431.58	433.4	2.57	0.59
10	435.22			
Change in Mobile Phase Concentration (71:29)				
10	525.58	484.19	58.54	12.09
10	442.79			
Change in Wavelength (232 nm)				
10	512.78	512.7	0.09	0.02
10	512.65			
Change in Wavelength (234 nm)				
10	386.68	386.75	0.09	0.02
10	386.81			

Table No-3.5 displayed the Robustness study of NIFE. Robustness studies of System were performed by changing the flow rate, M.P concentration and wavelength. The mean found were 514.55 and 385.93 for the flow rate of 0.6 ml/min and 0.8 ml/min respectively. The %RSD found were 0.13 and 0.23 for the flow rate of 0.6 ml/min and 0.8 ml/min respectively. The mean found were 433.4 and 484.19 for Mobile Phase Concentration (69:31) and Mobile Phase Concentration (71:29) respectively. The %RSD found were 0.59 and 12.09 for Mobile Phase Concentration (69:31) and Mobile Phase Concentration (71:29) respectively. The

mean found were 512.7 and 386.75 for Wavelength (232 nm) and Wavelength (234 nm) respectively. The %RSD found were 0.02 and 0.02 for Wavelength (232 nm) and Wavelength (234 nm) respectively. This study shows that system is robust and withstand by changing different aspects of system.

Table No: 3.6 Repeatability

Atenolol								
Sr No.	Conc.	Area I	Area II	Mean	Amt Found	% Amt Found	SD	%RSD
1	100	8695.45	8691.24	8693.35	99.40	99.40	2.98	0.03
Nifedipine								
	40	1681.07	1681.22	1681.15	39.98	99.95	0.11	0.01

Table No- 3.6 displayed the system suitability test (Repeatability) study of ATE and NIFE. The mean areas found were 8693.35 and 1681.15 for ATE and NIFE respectively. The % amount recovered was 99.40 and 99.95 for ATE and NIFE respectively.

Table No: 3.7 Assay of Atenolol

Conc.	Area	Amt Found	% Label Claim
75.00	6515.05	74.28	99.04
75.00	6510.04	74.23	98.97
Mean	6512.55	39.67	99.01
SD	3.54	0.04	0.01
%RSD	0.05	0.09	0.01

Table No: 3.8 Assay of Nifedipine

Conc.	Area	Amt Found	% Label Claim
30.00	1270.56	29.92	99.73
30.00	1274.86	30.03	100.10
Mean	1272.71	29.97	99.92
SD	3.04	0.08	0.02
%RSD	0.24	0.26	0.02

Table No- 3.7 displayed the assay of ATE. The mean area was found 6512.55. The average % recovered was 99.01 and %RSD was 0.01. Table No- 3.8 displayed the assay of NIFE. The mean area was found 1272.71. The average % recovered was 99.92 and %RSD was 0.02.

References

1. United State Pharmacopoeia -30, National Formulary - 25, 2007, By Authority of the United State Pharmacopoeial Convention, Inc. Prepared by the Council of Experts and Published the Board of Trustees: 1456, 2569.
2. A. Bright, T. S. Renuga Devi* and S. Gunasekaran 2010. Application of RP-HPLC and UV-Visible Spectroscopy for the Estimation of Atenolol and Verapamil in Tablets Before and After the Expiry Period, International Journal of ChemTech Research, 2(2): 865-870.
3. Lakshmi Narasimham Y.S.*, Barhate V.D. 2010. Development and validation of stability indicating UPLC method for the simultaneous determination of beta-blockers and diuretic drugs in pharmaceutical dosage forms, Journal of chemical metrology, 4(1):1-20.
4. S.Vidyadhara, RLC Sasidhar, B.Praveen Kumar, NT Ramarao and N.Sriharita. 2012, Method Development and Validation for Simultaneous Estimation of Atenolol and Nifedipine in Pharmaceutical Dosage Forms by RP-HPLC, Oriental journal of chemistry, 28(4):1691-1696.
5. Essam Ezzeldin et al 2014. Development and validation of LC/MS/MS method for the simultaneous determination of montelukast, gliclazide, and Nifedipine and its application to a pharmacokinetic study, 8(17):1-9.
6. M. S. Charde*, A. S. Welankiwar1 and R. D. Chakole 2014. Development of validated RP-HPLC method for the simultaneous estimation of Atenolol and chlorthalidone in combine tablet dosage form, International Journal of Advances in Pharmaceutics, 3(1):6-18.
7. *Sahaya Asirvatham, Neelam Sachin Kamble 2014. Reversed-Phase Liquid Chromatographic Method for Simultaneous Determination of Atenolol and Nifedipine in a Capsule Formulation, World Journal of Pharmaceutical Research, 3(9):1466-1475.
8. N.M. Kassab et al 2017. Development and Validation of an Isocratic HPLC Method for Simultaneous Determination of Quaternary Mixtures of Antihypertensive Drugs in Pharmaceutical Formulations, Acta Chromatographica, 29(2017)1:95-110.

9. Sojitra Rajanit*, Virani Paras, Hashumati Raj 2015. Development and Validation of First Order Derivative Spectrophotometric method for simultaneous estimation of Nifedipine and Metoprolol Succinate in Synthetic Mixture, International Journal of Pharma Sciences and Research, 6(2): 265-272.
10. Instrumental methods of Analysis, by H. Willard, L. Merritt, J. Dean, F. Settle, CBS Publishers and Distributors Pvt. Ltd, 7th editioin:580-610.