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Stability indicating RP-HPLC method for the determination of amlodipine besylate

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ABSTRACT

A simple, specific, sensitive, accurate, precise, economic & reproducible RP-HPLC method was developed for the amlodipine and validated. The proposed method utilises the Agilent technologies: Agilent 1260&1290 series. Column ZORBAX C18 100 x 4.6mm with 3.5microns particle size and a software of ECZ chrome elite, 0.2µ Millipore filter paper and by using optimum mobile phase methanol: acetonitrile: Water (80:10:10) at flow rate of 1.0ml/min at 35+/- 2^{o}c temperature. The eluent was detected at 230nm by using U.V detector. The retention time of Amlodipine besylate was found to be 1.5 min and is economically feasible. The linearity was observed at 2-50µ.g/ml. The mean recoveries were found to be 100±2%. The method was validated for system suitability, specificity, linearity, accuracy, precision, ruggedness, robustness, LOD & LOQ as per ICH guidelines. Limit of detection and Limit of quantification of the method was found to be 2.93µg/ml and 8µg/ml shows that the developed method has adequate sensitivity. (r^2 > 0.99), %R.S.D of precision was found to be <2. The developed method was used for the stability, forced degradation studies. It can be used in the quality control and in-vitro dissolution of the drug. The proposed method is precise, economic, with high sensitivity and less retention time.

Keywords: Amlodipine Besilate, LOD & LOQ

INTRODUCTION

Amlodipine besylate 4R, S 3- ethyl 5-methyl 2-(2- amino – ethoxy methyl)-4 - (2-chlorophenyl)-1,4 dihydro – 6 –methyl pyridine 3, 5 dicarboxylate mono benzene sulphonate is a potent long acting calcium channel blocking agent. It is widely used for the treatment of hypertension as well as the stable and variant angina. It is more effective than beta blocker in the treatment of variant angina because it prevents and reverses the coronary spasms resulting in increased blood flow myocardial oxygen supply. It inhibits selectively the arterial vascular smooth muscle cell proliferation resulting in prevention of the progressive narrowing of the arteries[1]

Hypertension is the most common cardiovascular disease found in 20-30% population of the developed world. According to WHO, hypertension is a state of body in which systolic blood pressure is 150mm Hg more and diastolic

pressure is 95mmHg or more. Hypertension may be classified into two types: Primary hypertension and Secondary hypertension. Primary hypertension is characterized by elevation of diastolic blood pressure, a normal cardiac output and an increase in peripheral resistance with a documented eitiology. Secondary hypertension is a type where etiology is known. It is secondary to some disorder.[9] Common disorders: Acute or chronic renal disease, Cushing's syndrome, Acromegali etc. (Joel GH, 1996 & Tripathi) [2]

Drug analysis plays an important role in the development of drugs their manufacture and therapeutic use pharmaceutical industries rely upon quantitative chemical analysis to ensure that the raw material used and the final product obtained meets the required specifications[3]. Because of therapeutic importance of AML, it is highly abundant drug; many methods have been developed for its determination of pharmaceutical dosage forms.[10] The aim of the present study is the stability indicating RP-HPLC method development and validation with less retention time, highly economic & with high sensitivity[5]

So here an attempt has been made to develop simple accurate rapid economic method for the determination of amlodipine by using HPLC method.[6]

AIM AND OBJECTIVE

According to the literature survey it was found that various analytical strategies have been used for the qualitative and quantitative estimation of Amlodipine besylate in pharmaceutical preparations and stability indicating methods by various analytical strategies have been used for the qualitative non-aqueous titration, voltammetry and Reverse phase-high performance liquid chromatography (RP-HPLC) [7].

In view of the need for a suitable method for routine analysis, attempts are being made to develop and validate simple, precise and accurate analytical method for the determination of Amlodipine besylate. For the determination of Amlodipine besylate HPLC method was adopted as an analytical tool to study the influence of different chromatographic conditions and to ensure that the compound of interest was well-separated in a faster elution time compared to previous methods. In light of these considerations, this work aimed to develop a new, simple, specific, accurate, viable and precise stability-indicating.

Validation of the method was done in accordance with ICH guidelines. The method was validated for parameters like accuracy, linearity, precision, specificity, sensitivity, robustness and system suitability. The proposed method may be suitable for the analysis in pharmaceutical quality control laboratories & also in in-vitro dissolution of the drug products.[8]

Objective of the proposed work is to

- Develop new, simple, sensitive, accurate and economical analytical method for the determination of Amlodipine besylate.
- Validate the proposed method in accordance with ICH guidelines for the intended analytical application.

METHODOLOGY

Determination of Absorption Maxima by UV-Visible Spectrophotometry

Preparation of Stock solution:

Accurately weighed and transferred about 10 mg of Amlodipine Besylate into a 10 ml volumetric flask, Dissolve the drug in 10 ml of given solvent system (methanol, acetonitrile, water+ methanol) gives the solution of 1000µg/ml

The solution was scanned in the range of 200-400 nm and from the spectrum, the λ_{max} of Amlodipine was found to be 230 nm.

Assay of the sample preparations

Preparation of solutions

Preparation of mobile phase: The mobile phase consisted of filtered and degassed mixture methanol: acetonitrile: water in the ratio (80:20:10).

Preparation of standard stock solution of Amlodipine besylate

10 mg of standard amlodipine was weighed and transferred to a 10 ml volumetric flask and dissolved in mobile phase with required quantities in proportions and then kept in ultrasonicator for 5min. the volume was made upto the mark with methanol to obtain final concentration 1000µ.g/ml.

Analysis of the marketed formulation (stamlo)

Twenty tablets were weighed and their average weight was determined and finely powdered. The powder equivalent to 5mg of AML was accurately weighed and transferred to 100ml volumetric flask and dissolved in 50ml of methanol and the flask was kept in ultrasonicator for 10 min. The flask was shaken and the volume was made upto the mark with the methanol to give a solution of 50µg/ml of AML. The solution was filtered through 0.2µ Millipore filter paper.

0.5ml of aliquot was pipetted out and transferred to a volumetric flask. The volume was made upto the mark with methanol to obtain a solution with final concentration 50µg/ml.

A 20µL volume of sample mix was injected into the sample injector of HPLC system and the chromatogram of blank, test and standard was recorded.

RESULTS

Area % Report

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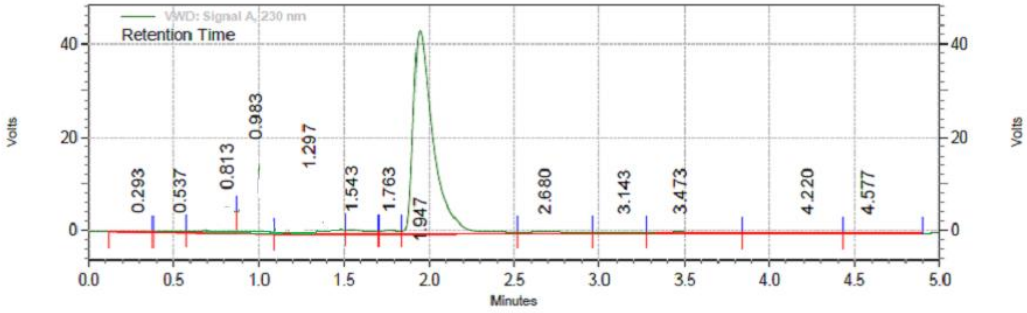

VWD: Signal A, 230 nm Results

Retention Time	Area	Area %	Height	Height %
0.293	21421	0.21	2261	0.17
0.537	52141	0.50	5573	0.42
0.813	650876	6.30	94445	7.07
0.983	1712817	16.59	276052	20.67
1.297	1094261	10.60	168199	12.59
1.543	154964	1.50	16626	1.24
1.763	103490	1.00	13996	1.05
1.947	5915483	57.28	730739	54.71
2.680	168606	1.63	7380	0.55
3.143	95752	0.93	5304	0.40
3.473	161765	1.57	6230	0.47
4.220	135107	1.31	5191	0.39
4.577	60629	0.59	3578	0.27
Totals	10327312	100.00	1335574	100.00

Observation

The retention time of Amlodipine besylate was 1.947 min. The peak was sharp with broad area & with no interference from other peaks .The system suitability parameters were passed.

Conclusion

The tailing factor was < 2.0, Resolution was optimum and Plate count was >2000, so this method is considered as the optimized method.

Chromatograms of Amlodipine besylate

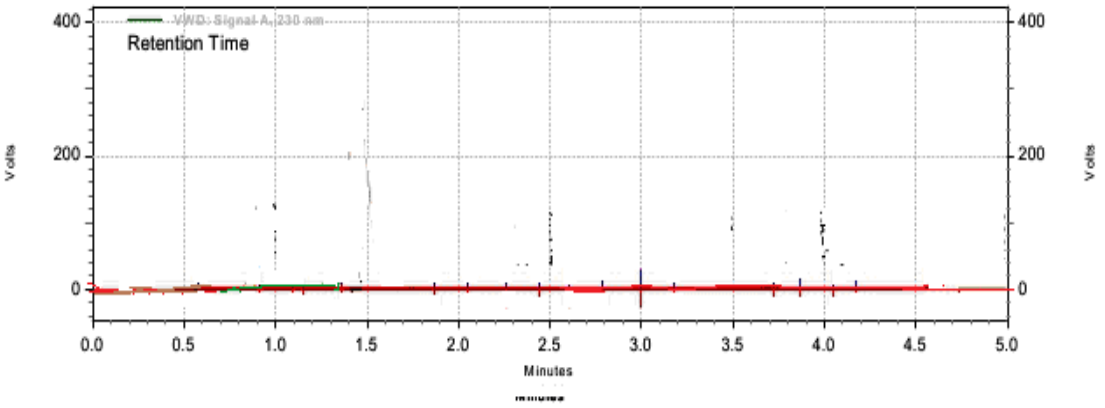

Fig: 1 Blank Chromatogram of Amlodipine besylate

Area % Report

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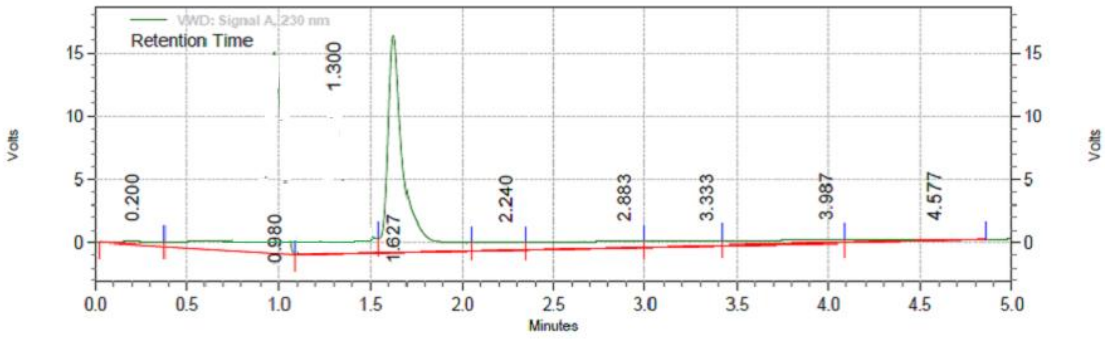

VWD: Signal A, 230 nm Results

Retention Time	Area	Area %	Height	Height %
0.200	61242	1.01	3155	0.41
0.980	1906970	31.45	267226	34.38
1.300	1356642	22.37	190347	24.49
1.627	1820865	30.03	287704	37.01
2.240	173204	2.86	9635	1.24
2.883	314650	5.19	7487	0.96
3.333	157265	2.59	5995	0.77
3.987	179319	2.96	3963	0.51
4.577	93179	1.54	1865	0.24
Totals	6063336	100.00	777377	100.00

Fig: 2 Chromatogram of Amlodipine besylate 2ug/ml

Area % Report

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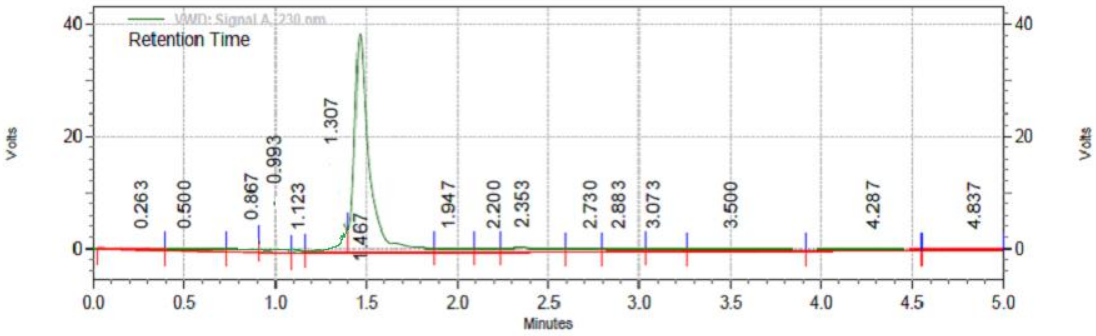

VWD: Signal A, 230 nm Results

Retention Time	Area	Area %	Height	Height %
0.263	58684	0.80	3792	0.32
0.500	123473	1.69	5408	0.46
0.867	221483	3.03	37067	3.12
0.993	786148	10.75	148579	12.50
1.123	24530	0.34	8025	0.68
1.307	1452719	19.86	262653	22.10
1.467	3607092	49.31	652652	54.92
1.947	139682	1.91	11451	0.96
2.200	89491	1.22	10380	0.87
2.353	196763	2.69	11593	0.98
2.730	92496	1.26	8515	0.72
2.883	118086	1.61	9071	0.76
3.073	87024	1.19	7327	0.62
3.500	176801	2.42	5677	0.48
4.287	115758	1.58	4760	0.40
4.837	24616	0.34	1506	0.13
Totals	7314846	100.00	1188456	100.00

Fig: 3 Chromatogram of Amlodipine besylate 5ug/ml

Area % Report

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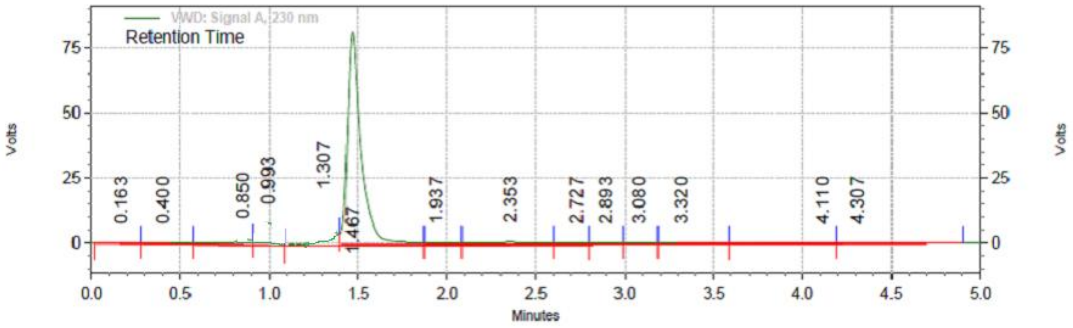

VWD: Signal A, 230 nm Results

Retention Time	Area	Area %	Height	Height %
0.163	61436	0.52	4700	0.25
0.400	139661	1.18	7717	0.41
0.850	385521	3.27	46107	2.42
0.993	882217	7.47	143889	7.56
1.307	1412024	11.96	242623	12.74
1.467	7446749	63.08	1375513	72.24
1.937	198960	1.69	16172	0.85
2.353	430395	3.65	16620	0.87
2.727	129479	1.10	11903	0.63
2.893	122820	1.04	11049	0.58
3.080	114078	0.97	10032	0.53
3.320	182260	1.54	8413	0.44
4.110	188062	1.59	5034	0.26
4.307	112032	0.95	4435	0.23
Totals	11805694	100.00	1904207	100.00

Fig: 4 Chromatogram of Amlodipine besylate 10ug/ml

Area % Report

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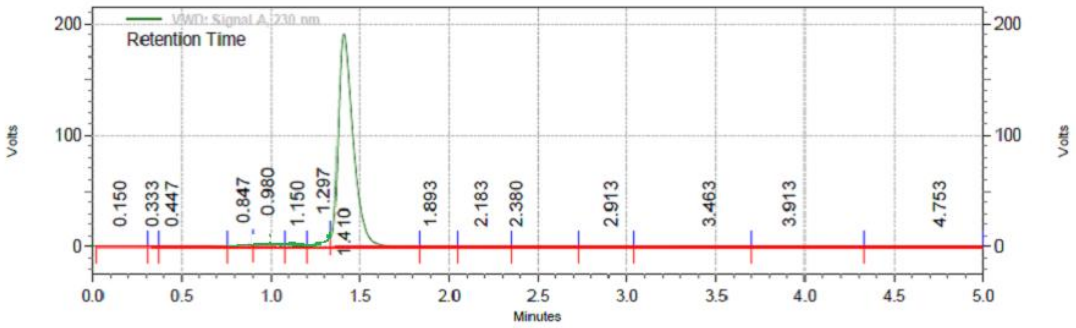

VWD: Signal A, 230 nm Results

Retention Time	Area	Area %	Height	Height %
0.150	38326	0.17	2269	0.06
0.333	15013	0.07	4086	0.11
0.447	156512	0.69	5671	0.15
0.847	320117	1.41	79741	2.10
0.980	1057960	4.67	207644	5.48
1.150	55692	0.25	10665	0.28
1.297	878263	3.88	211433	5.58
1.410	23043620	84.15	3215481	84.86
1.893	148559	0.66	12978	0.34
2.183	185265	0.82	11142	0.29
2.380	186141	0.82	9141	0.24
2.913	126199	0.56	6724	0.18
3.463	229083	1.01	5910	0.16
3.913	137652	0.61	4458	0.12
4.753	52685	0.23	1683	0.04
Totals	22631087	100.00	3789026	100.00

Fig: 5 Chromatogram of Amlodipine besylate 30ug/ml

Area % Report

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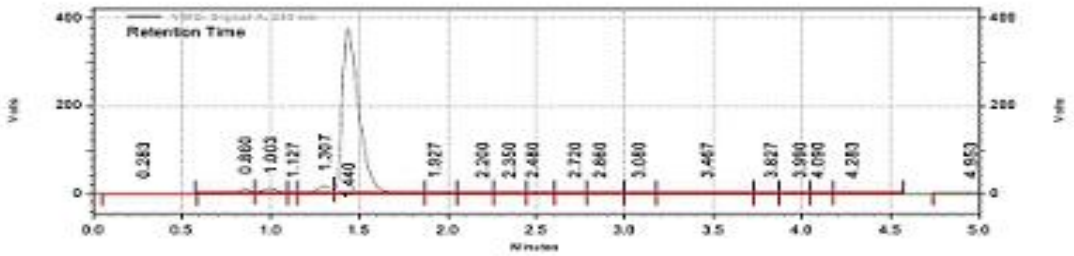

VWD: Signal A, 230 nm Results

Retention Time	Area	Area %	Height	Height %
0.283	30918	0.07	2291	0.03
0.860	587891	1.33	169008	2.42
1.003	960351	2.17	171863	2.46
1.127	17074	0.04	6064	0.09
1.307	1392392	3.15	272167	3.89
1.440	40740195	92.04	6321783	90.39
1.927	93392	0.21	10938	0.16
2.200	70306	0.16	6027	0.09
2.350	64615	0.15	7170	0.10
2.480	35254	0.08	3993	0.06
2.720	38182	0.09	4056	0.06
2.860	39546	0.09	3937	0.06
3.080	25577	0.06	2662	0.04
3.467	84810	0.19	3632	0.05
3.827	13028	0.03	1526	0.02
3.990	20267	0.05	2325	0.03
4.090	14102	0.03	2116	0.03
4.283	28327	0.06	2160	0.03
4.953	5167	0.01	444	0.01
Totals	44261394	100.00	6994162	100.00

Fig: 6 Chromatogram of Amlodipine besylate 50ug/ml

Multiple calibration graph of amlodipine besylate

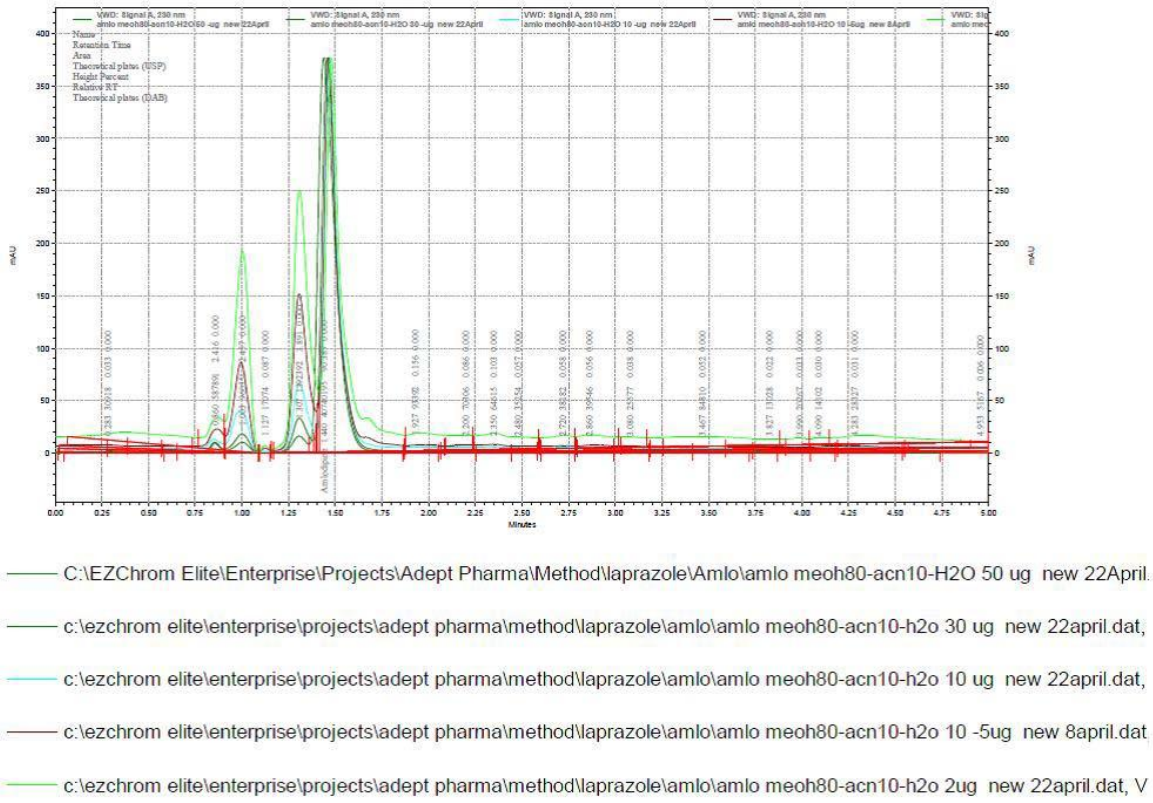

Fig: 7 Multiple Calibration Graph

Assay

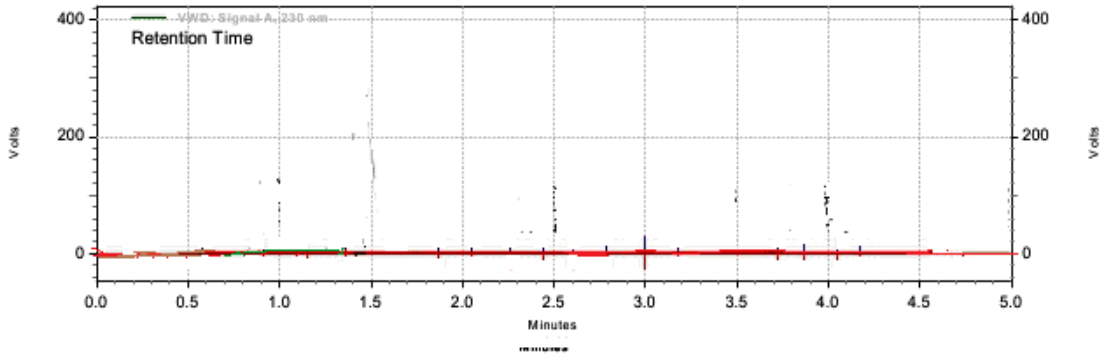

Fig: 8 Chromatogram of blank

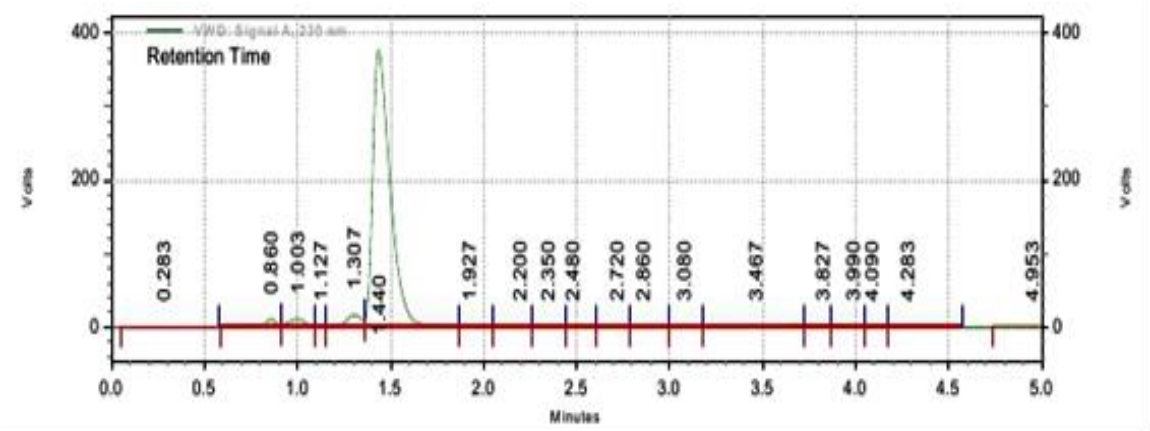

Fig: 9 Chromatogram of Test

Peak name	Retention time	Peak Area	% Area	USP Tailing	USP Plate count
Amlodipine besylate	1.440 min.	845634	100.00	1.08	2538.9

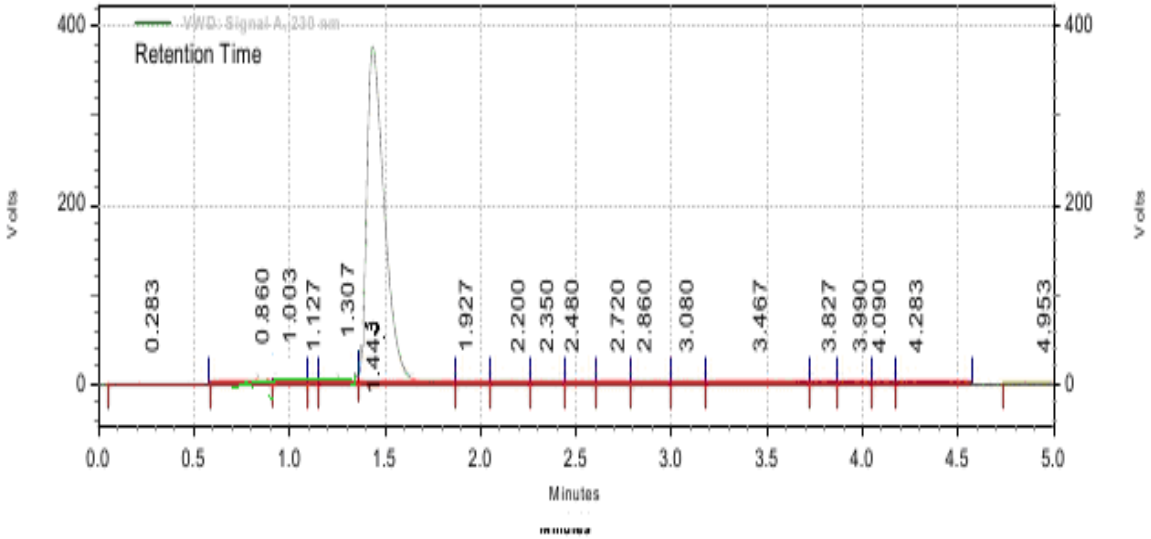

Fig: 10 Chromatogram of standard preparation

Peak name	Retention time	Peak Area	% Area	USP Tailing	USP Plate count
Amlodipine besylate	1.443 min.	862111	100.00	1.07	2541.1

Observation

The retention time of Amlodipine besylate standard peak was 1.443 min.

The retention time of Amlodipine besylate was 1.440 min.

The system suitability parameters were evaluated from the standard chromatograms

System suitability parameters

%RSD of Peak areas	:	NMT 2.0
Tailing factor	:	NMT 2.0
Theoretical Plates	:	NLT 2000

Degradation studies

Area % Report

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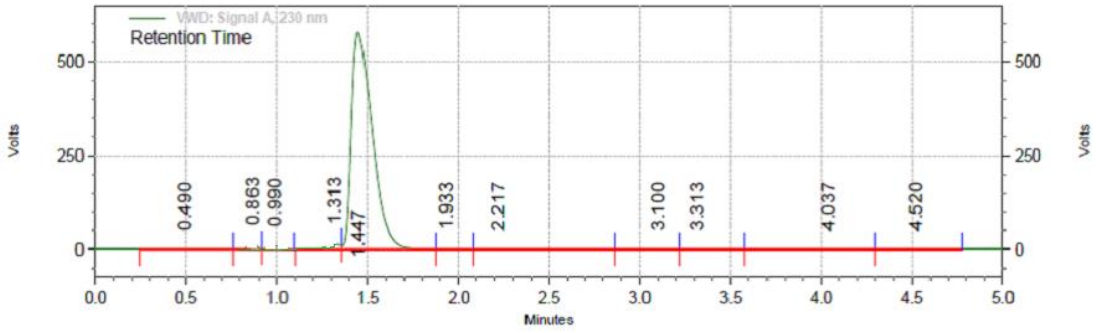

VWD: Signal A, 230 nm Results

Retention Time	Area	Area %	Height	Height %
0.490	133809	0.16	4674	0.04
0.863	904171	1.11	241732	2.32
0.990	992297	1.21	178080	1.71
1.313	1359883	1.66	256030	2.46
1.447	77138164	94.32	9680291	92.87
1.933	230974	0.28	23805	0.23
2.217	541681	0.66	18201	0.17
3.100	156834	0.19	7905	0.08
3.313	112100	0.14	5977	0.06
4.037	168377	0.21	4139	0.04
4.520	49129	0.06	2241	0.02
Totals	81787419	100.00	10423075	100.00

Fig: 11 Chromatogram of Acidic Hydrolysis.

Area % Report

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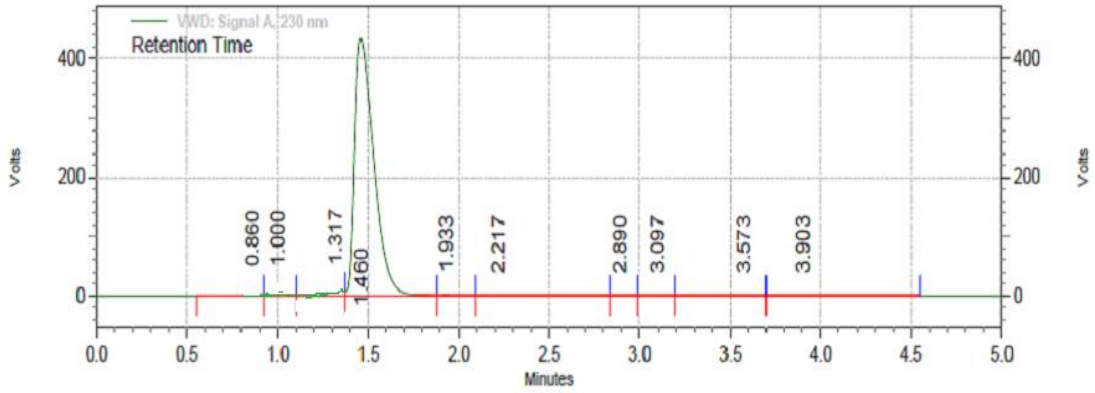

VWD: Signal A, 230 nm Results

Retention Time	Area	Area %	Height	Height %
0.860	697493	1.23	182302	2.31
1.000	891154	1.57	155774	1.97
1.317	1166047	2.06	210930	2.67
1.460	52947841	93.44	7287334	92.39
1.933	177205	0.31	17569	0.22
2.217	395408	0.70	14017	0.18
2.890	50313	0.09	5578	0.07
3.097	63507	0.11	5375	0.07
3.573	137112	0.24	4703	0.06
3.903	136352	0.24	4207	0.05
Totals	56662432	100.00	7887789	100.00

Fig: 12 Chromatogram of Basic Hydrolysis.

Area % Report

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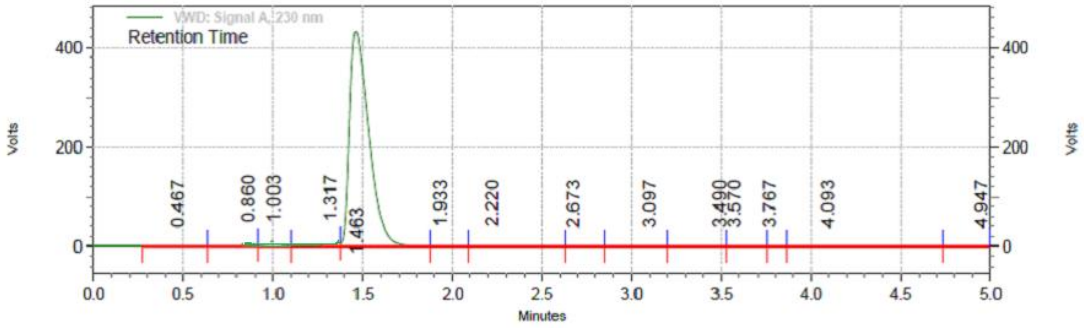


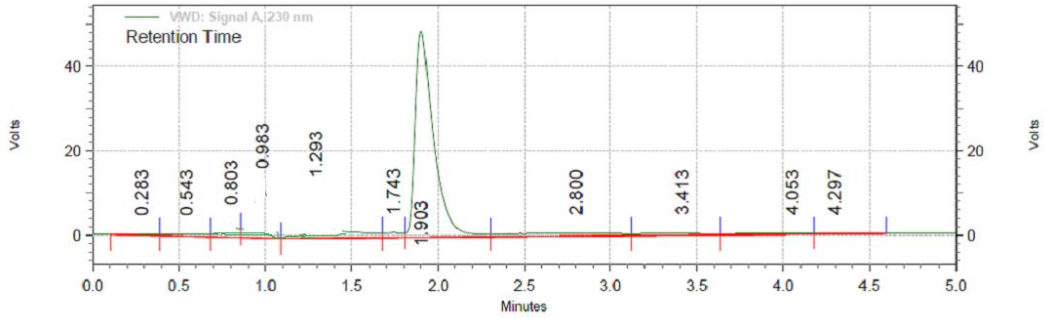
VWD: Signal A, 230 nm Results

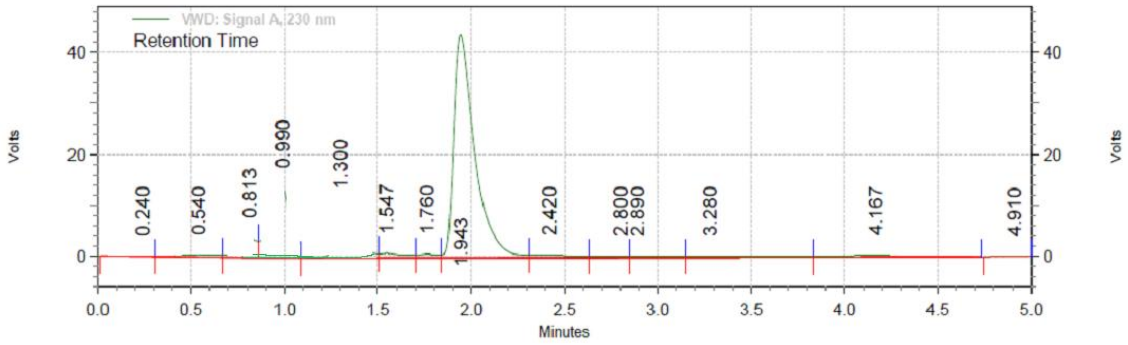
Retention Time	Area	Area %	Height	Height %
0.467	58756	0.10	3112	0.04
0.860	733590	1.29	184613	2.35
1.003	974530	1.71	174001	2.21
1.317	1153457	2.02	202239	2.57
1.463	53030970	93.07	7239724	92.03
1.933	181395	0.32	18272	0.23
2.220	331539	0.58	14592	0.19
2.673	89758	0.16	7069	0.09
3.097	124150	0.22	6224	0.08
3.490	97239	0.17	5029	0.06
3.570	59965	0.11	4833	0.06
3.767	22894	0.04	3609	0.05
4.093	117606	0.21	3181	0.04
4.947	4726	0.01	271	0.00
Totals	56980575	100.00	7866769	100.00

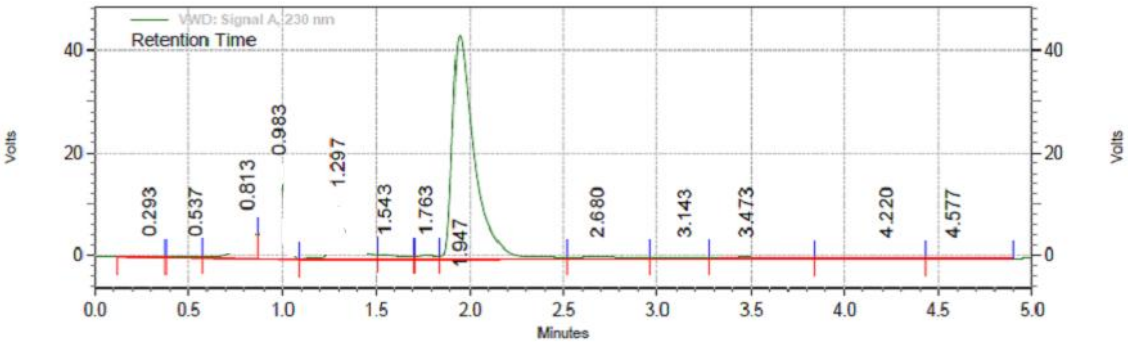
Fig: 13 Chromatogram of Oxidation.

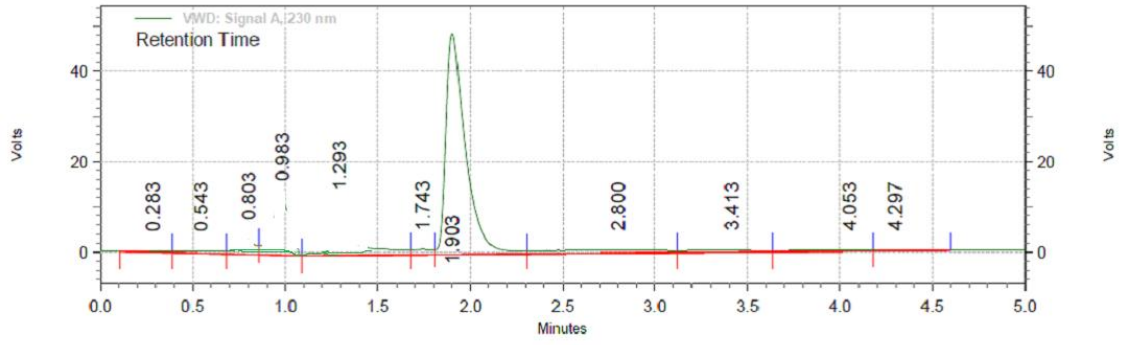
METHOD VALIDATION

System suitability parameters




VWD: Signal A, 230 nm
Retention Time
Volts
Minutes
0.240
0.540
0.813
0.990
1.300
1.547
1.760
1.943
2.420
2.800
2.890
3.280
4.167
4.910


VWD: Signal A, 230 nm
Retention Time
Volts
Minutes
0.293
0.537
0.813
0.983
1.297
1.543
1.763
1.947
2.680
3.143
3.473
4.220
4.577


VWD: Signal A, 230 nm
Retention Time
Volts
Minutes
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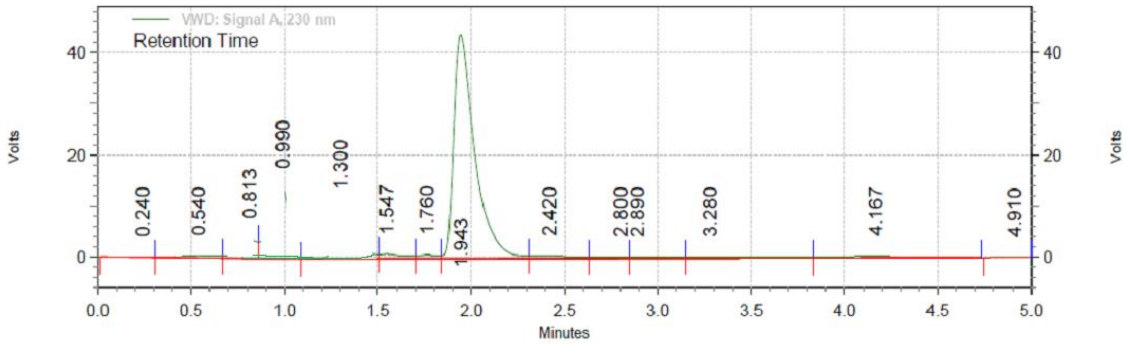
0.543
0.803
0.983
1.293
1.743
1.903
2.800
3.413
4.053
4.297


VWD: Signal A, 230 nm
Retention Time
Volts
Minutes
0.240
0.540
0.813
0.990
1.300
1.547
1.760
1.943
2.420
2.800
2.890
3.280
4.167
4.910

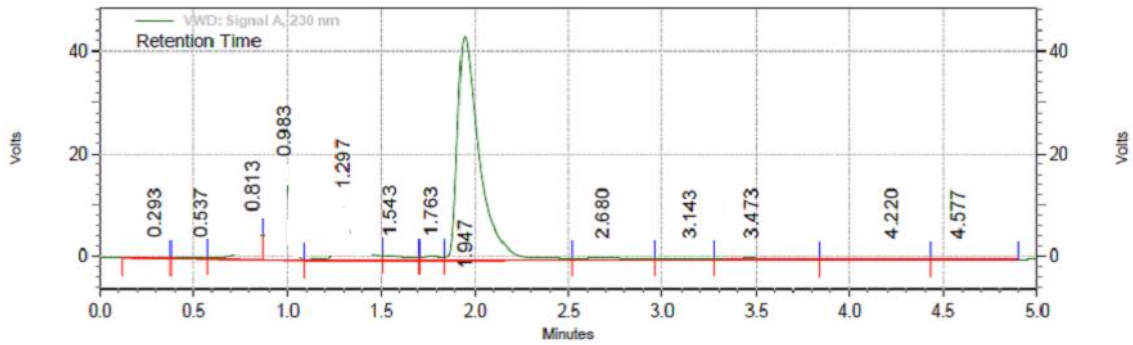


Fig: 14 Chromatograms of system suitabililty

Table: 1 Acceptance criteria for Amlodipine besylate.

System suitability parameter	Acceptance criteria
Relative Standard Deviation (%R.S.D)	% R.S.D should be <2
Tailing factor	T should be <2
Theoretical plate	In general N should be >2000

Table: 2 System suitability tests for Amlodipine besylate.

S.No	Retention time Mean±S.D	%R.S.D	Tailing factor Mean±S.D	%R.S.D	Theoretical plate Mean±S.D	%R.S.D
1	1.947±0.0009	0.04	1.16±0.002	0.17	2725±16	0.6
2	1.943±0.0008	0.04	1.10±0.002	0.18	2719±30	1.1
3	1.903±0.0008	0.04	1.60±0.002	0.12	2724±20	0.7
4	1.943±0.0008	0.04	1.10±0.002	0.18	2719±30	1.1
5	1.903±0.0008	0.04	1.60±0.002	0.12	2724±20	0.7
6	1.947±0.0009	0.04	1.16±0.002	0.17	2725±16	0.6

Observation

- **%** R.S.D for six replicate injections of peak area for Amlodipine Besylate standard preparation was found to be less than 1%
- Tailing factor for six Amlodipine Besylate peaks was found to be less than 2%
- Number of Theoretical plates for six Amlodipine Besylate was found to be more than 2000

Inference

All the system suitability parameters were satisfied, thus system suitability test was passed

Linearity

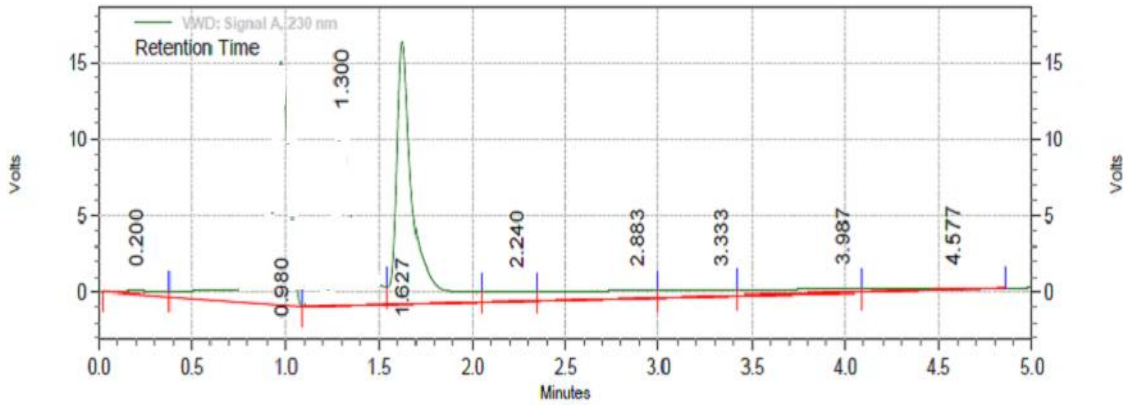


Fig: 15 Chromatogram of linearity 2µg/ml

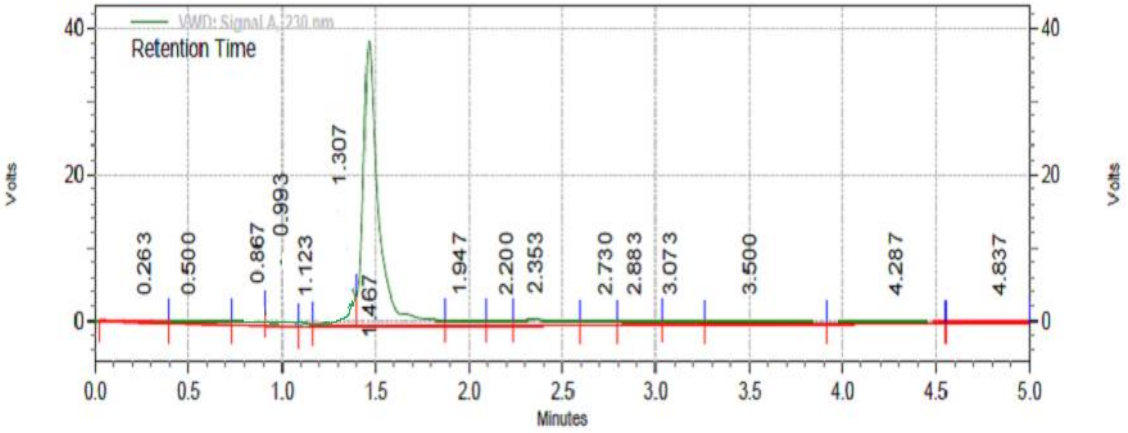


Fig: 16 Chromatogram of linearity 5µg/ml

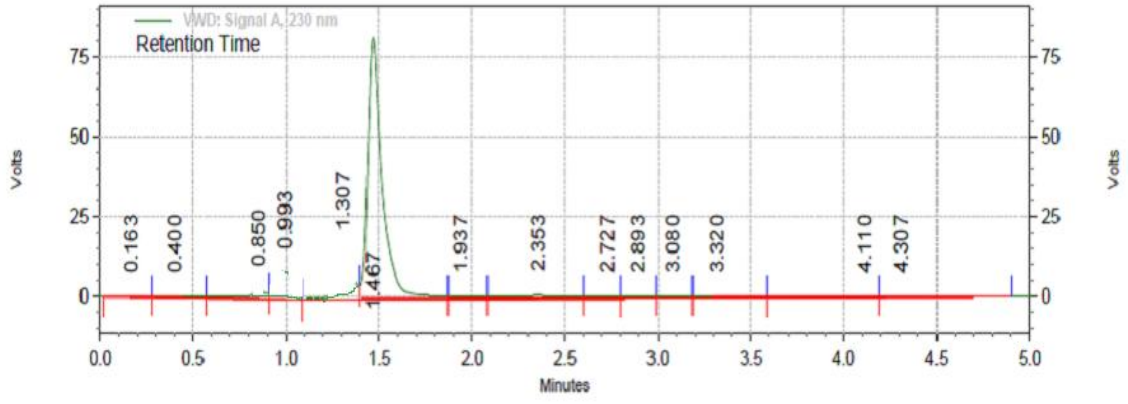


Fig: 17 Chromatogram of linearity 10µg/ml

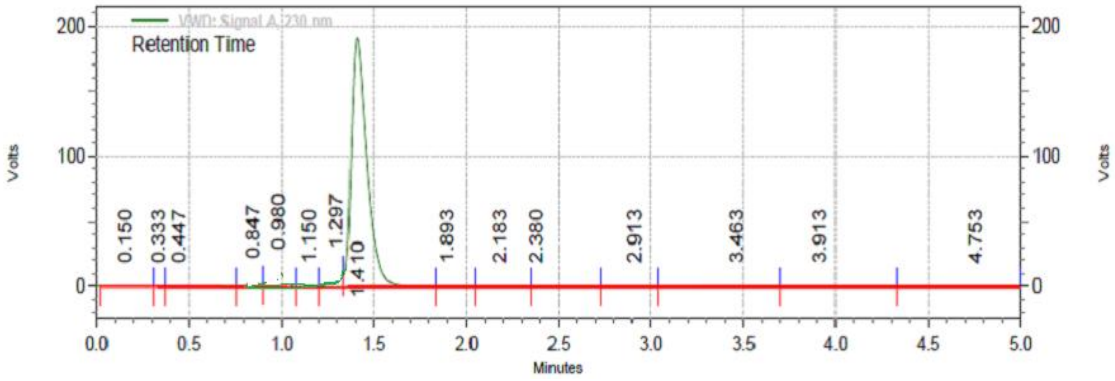


Fig: 18 Chromatogram of linearity 30µg/ml

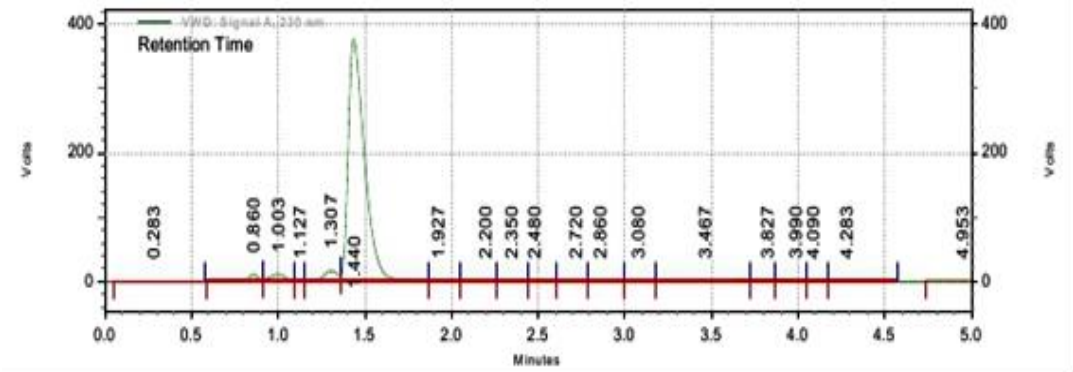


Fig: 19 Chromatogram of linearity 50 µg/ml

Linearity curve of Amlodipine Besylate

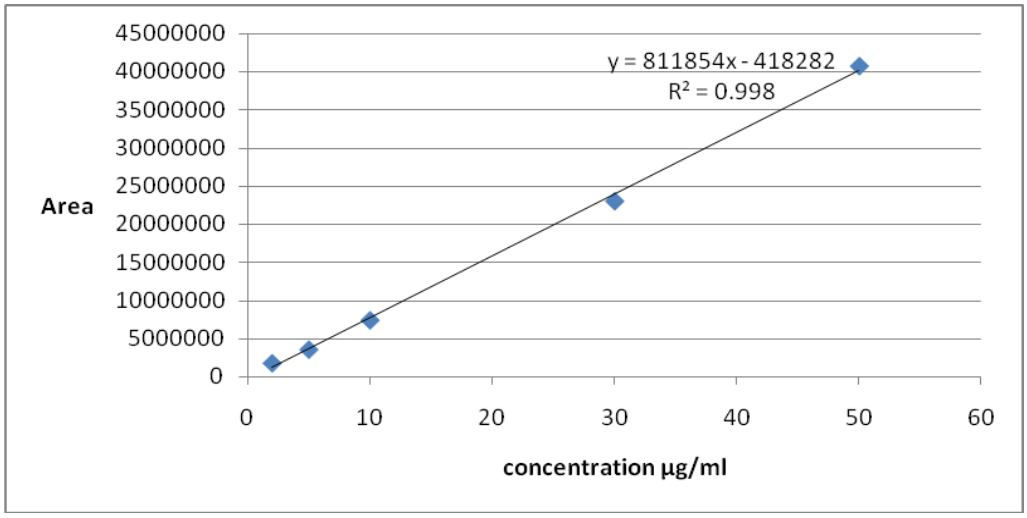


Fig: 20 Linearity curve

Table: 3 Peak areas for Linearity solutions of Amlodipine Besylate.

S.no	Concentration	Area
1	2	1820865
2	5	3607092
3	10	7446749
4	30	23043620
5	50	40740195

Linearity

Slope: 811855
Intercept: 418282
Linearity range: 2-50
Correlation coefficient: 0.9992

Observation

The straight line equation for the calibration curve was found to be y=811855x + 418282

Correlation co-efficient (r^2) was found to be 0.9992

Inference

Linearity was observed with in the range of 2-50µg/ml for Amlodipine Besylate and r^2 was >0.99 hence the method is said to be linear.

Precision

Intra-day Precision

Table: 4 Results of Intra-day Precision

Concentration	Set-1	Set-2	Set-3	Mean±S.D	%R.S.D
2	1820865	1803259	1818923	1814349±9653.18	0.5
10	7446749	7425049	7432058	7434618.67±11074.3	0.14
50	40740195	40405829	40655591	40600538.3±173848.3	0.4

Inter-day precision

Table: 5 Results of inter-day precision.

Concentration	Set-1 (Day -1)	Set-2 (Day 2).	Set-3 (Day 3)	Mean ±S.D	%R.S.D
2	1820284	1818695	1818258	1819079±1066.18	0.05
10	7429054	7426086	7431177	7428772±2557.16	0.03
50	40646919	40644944	40641979	40644614±2486.47	0.01

Observation

The % R.S.D of peak areas of Amlodipine Besylate solution were found to be in the range of 0.14 – 0.5 and 0.01 -0.05 for intra-day and inter-day precision respectively.

Inference

The %R.S.D values were found to be below 2% hence the method is said to be precise.

Accuracy

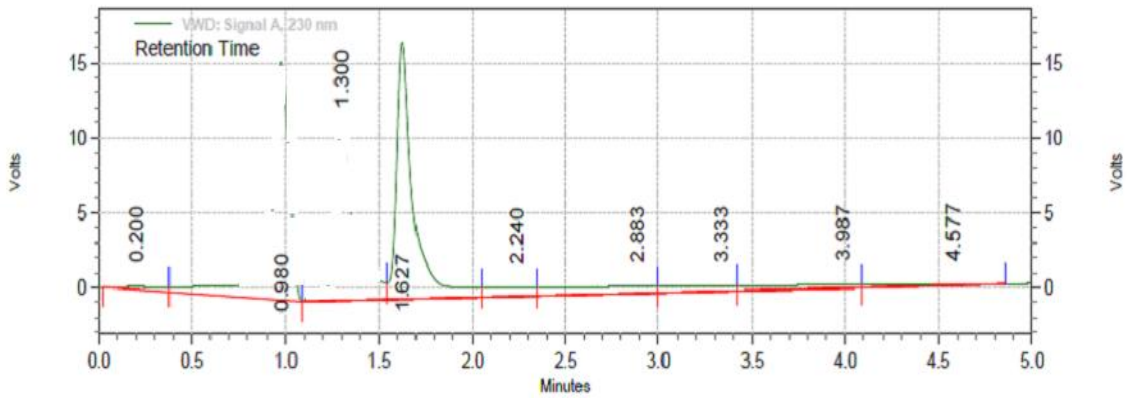


Fig: 21 Chromatogram of accuracy 2µg/ml

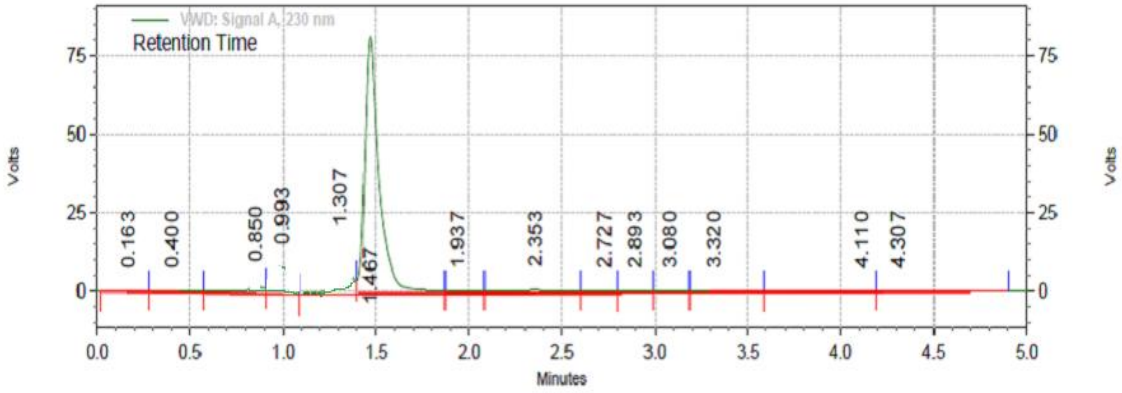


Fig: 22 Chromatogram of accuracy 10µg/ml

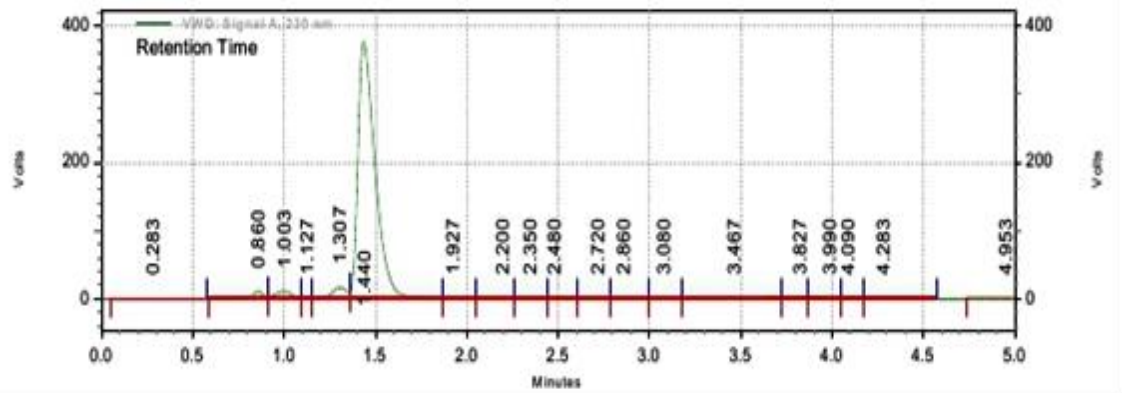


Fig: 23 Chromatogram of accuracy 50 µg/ml

Table: 6 % Recovery data for Amlodipine besylate.

Amount Present	Amount Added	Drug recover--ed	% recove--ry	Area	Mean	S.D	%R.S.D
2	50	36839	100+0.08	42597899 42586492 42498967	42561119.3	54126.8	0.13
10	30	12320	100+0.02	48199264 48096579 48278911	48191584.67	91408.25	0.189
50	50	12734	100+0.02	63796549 63768954 63745678	63770393.67	25466	0.03

Observation

The recovery results indicate that the test method has an acceptable level of accuracy

Inference

The mean% recovery of Amlodipine Besylate was found to be in the range of 100+0.02 to100+0.08 hence the method is said to be accurate.

The %R.S.D values were found to be below 2% hence the method is said to be accurate.

Specificity

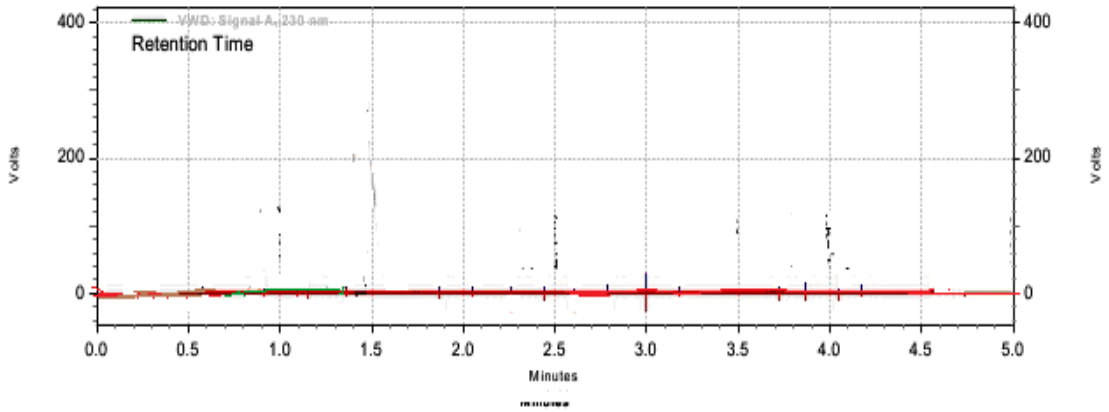


Fig: 24 Chromatogram of blank

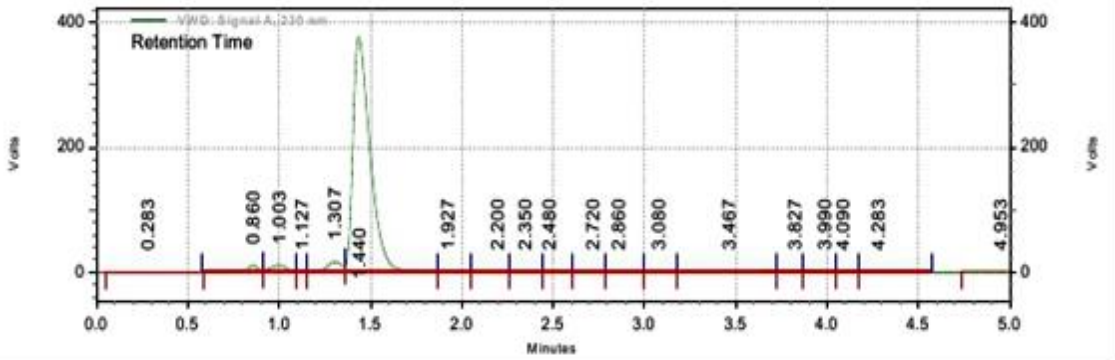


Fig: 25 Chromatogram of test preparation

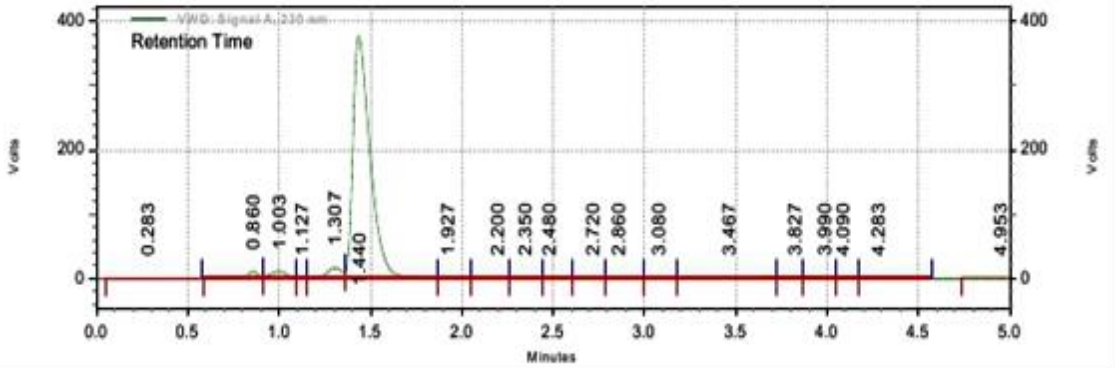


Fig: 26 Chromatogram of standard preparation

Observation

No interference was observed in the blank chromatogram at the retention times of standard and sample preparation of Amlodipine Besylate.

Interference

The method is found to be specific for the estimation of Amlodipine Besylate.

Limits of Detection

LOD µg/ml = 3.3×σ/S

From the results it was observed that

σ = 721807, S= 811855

Where σ is the standard deviation of the response and S is the slope of the calibration.

Result

The Limits of detection for Amlodipine Besylate was found to be 2.93µg/ml

Limits of Quantitation

LOQ µg/ml = 10×σ/S

From the results it was observed that

σ = 721807, S= 811855

Where σ is the standard deviation of the response and S is the slope of the calibration.

Result

The Limits of quantitation for Amlodipine Besylate was found to be 8µg/ml

Robustness

Effect of variation in mobile phase

Table: 7 Data for variation in mobile phase

Mobilephase ACN %level	Retention	%R.S.D	Tailing factor	%R.S.D	Theoretical plate	%R.S.D
10%	1.947±0.00957	0.4	1.16±0.002	0.17	2725±16	0.5
20%	1.903±0.00957	0.4	1.67±0.002	0.19	2724±20	0.7
40%	1.787±0.0008	0.3	1.67±0.002	0.19	2719±30	1.1
10.5%	1.947±0.00957	0.4	1.16±0.002	0.17	2725±16	0.5
9.5%	1.947±0.00957	0.4	1.16±0.002	0.17	2725±16	0.5

Observation

The %R.S.D values were found to be within the limits

Inference

The %R.S.D values were found to be below 2%, hence the method is said to be robust.

Effect of variation of flow rate

Table: 8 Data for variation in flow rate

Flowrate	Retention time	%R.S.D	Tailing factor	%R.S.D	theoretical plate	%R.S.D
1ml/min	1.947±0.00957	0.4	1.16±0.002	0.17	2725±16	0.5
0.9ml/min	1.9473±0.00957	0.4	1.163±0.002	0.17	2724±7	0.2
1.1ml/min	1.9469±0.00957	0.5	1.161±0.002	0.17	2726±20	0.7

Observation

The %R.S.D values were found to be within the limits

Inference

The %R.S.D values were found to be below 2%, hence the method is said to be robust.

Effect of variation of temperature

Table: 9 Data for variation in Temperature

Temperature	Retention time	%R.S.D	Tailing factor	%R.S.D	theoretical plate	%R.S.D
35◦c	1.947±0.00957	0.4	1.16±0.002	0.17	2725±16	0.5
32◦c	1.947±0.00957	0.4	1.16±0.002	0.17	2725±16	0.5
38◦c	1.947±0.00957	0.4	1.16±0.002	0.17	2725±16	0.5

Observation

The %R.S.D values were found to be within the limits

Inference

The %R.S.D values were found to be below 2%, hence the method is said to be robust.

CONCLUSION

For routine analytical estimation of the drugs, it is desirable to establish methods capable of analyzing huge number of samples in a short period with good robustness, accuracy and precision.

The objective of the proposed work was to develop an appropriate method for the determination of stability indicating Amlodipine

Besylate by RP-HPLC and to validate the developed method according to ICH guideline and applying the same method for use in the analysis of quality control samples in pharmaceutical industry

As there is no official method available in IP, BP &USP for the estimation of Amlodipine, so attempts were made to develop by which the amount of drug presents in the sample can be quantified,With high sensitivity& low retention time.

In the proposed RP-HPLC method, the parameters were optimized to obtain suitable conditions for the analysis of Amlodipine besylate. Initially various mobile phase compositions were tried. Mobile phase and flow rate selection was based on peak parameters (fronting, tailing, theoretical plates, capacity or symmetry factor), run time and resolution. The method with methanol: water: acetonitrile (80:10:10) mobile phase at flow rate of 0.1 ml/min was found to be optimum.

The optimum wavelength for detection was 230 nm at which better detector response for Amlodipine besylate was obtained. The retention time for Amlodipine was found to be 1.5min. To ascertain the effectiveness of the system used, system suitability tests were carried out on freshly prepared stock solution and the results met with acceptance criteria. The calibration was linear at concentration of 2-50µg/ml with correlation coefficient of 0.9992.

No interference was seen due to mobile phase solvents at the retention time of Amlodipine which confirms that the method was specific. The limit of detection and limit quantitation for Amlodipine were found to be 2.93µg/ml &8µg/ml which specify the method's sensitivity.

The values of %R.S.D 2% indicate that the method was precise. The mean recoveries were found to be in the range of 100+0.02 to100+0.08 which indicates that the method is accurate. Method robustness was calculated by alternation of flow rate and mobile phase composition. The method was robust as the %R.S.D was below 2.0%.

The proposed method was validated in accordance with ICH parameters and the applied for analysis of the same in marketed formulations.

Finally, it can be concluded that the proposed method was found to be accurate, precise, robust, specific and sensitive and can be successfully applied for the analysis of Amlodipine besylate.

REFERENCES

[1]. Sharma BK (2002). *Instrumental Methods of Chemical Analysis*. 21st ed. Meerut: Goel Publishing House, p. 3-5.

[2]. Skoog DA, West DM and Holler FJ (1996). *Fundamentals of Analytical Chemistry*. 7th ed. Philadephia: Saunders College Publishing. p. 1-3.

[3]. Beckett AH and Stenlake JB (2004). *Practical Pharmaceutical Chemistry*. Part 2. New Delhi: CBS Publishers and Distributors. p. 282-283

[4]. Willard HH, Merritt LL, Dean JA and Settle FA (2001). *Instrumental Methods of Analysis*. 7th ed. Delhi: CBS Publishers and Distributors. p.3-5.

[5]. Kasture AV, Mahadik KR, Wadodker SG, More HN, *Instrumental methods of Pharmaceutical analysis*, 14th ed. Pune : Nirali Prakashan. p-48.

[6]. Skoog DA, Holler FJ, Timothy A and Nieman NW (2004). *Principle of Instrumental Analysis*. 5th ed. Bangalore: Eastern Press. p. 1-2, 678-830.Scott RPW (2003). *Technique and Practice of chromatography*. Marcel Dekker: Vol. 70. New York: p. 1-12.

[7]. Jeffery GH, Basset J, Mendham J and Denney RC (1996). *Vogel's textbook of Quantitative Chemical analysis*. 5th ed. England: Longman Publication. p. 647-649.

[8]. Connors KA (1999). *A textbook of Pharmaceutical Analysis*. 8th ed. New York: Wiley-Interscience. p. 408-421.

[9]. Hamilton RJ and Sewell PA (1982). *Introduction to HPLC*. 2nd ed. London: Chapman and Hall. p. 189.

[10]. David CL, Michael Webb (1994). *Handbook of Pharmaceutical Analysis.* London: Blackwell publishing, p.2-4.