Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2015, 7 (10):20-29
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Validated RP- HPLC method for simultaneous determination of amlodipine and metoprolol in bulk drug and pharmaceutical formulations

Ravi Challa and N. V. S. Naidu*

Department of Chemistry, S.V.University, Tirupati, A.P., India

---

ABSTRACT

A simple stability indicating high performance liquid chromatographic method has been developed for the simultaneous determination of amlodipine besylate in combination with metoprolol succinate using reverse phase Hypersil C_{18} column (150x4.6, 5µ) with UV detection at 230 nm. The mobile phase consisting of potassium dihydrogen phosphate buffer and acetonitrile in a ratio of 65:35 (v/v) P^H was adjusted to 6.8±0.1 and at a flow rate of 1.0 mLmin^{-1}.The method was linear over the concentration range for amlodipine 2.5-15 µgml^{-1} and for metoprolol 12-75 µgml^{-1}. The recoveries of active pharmaceutical ingredient (API) amlodipine besylate and metoprolol succinate were found to be in the range of 100.10 – 101.16% and 98.50-100.02% respectively. The method was validated and was successfully employed for the routine quantitative analysis of pharmaceutical formulations containing amlodipine besylate and metoprolol succinate in combined tablet dosage form.

Keywords: Amlodipine, Metoprolol, RP-HPLC, Validation and pharmaceutical formulations

---

INTRODUCTION

High blood pressure can be treated with number of drugs depending upon the causes which are responsible for it. It is increasingly appreciated that the elusive goal of a 'normal' blood pressure is achieved only if multi-drug therapy is employed [1]. Amlodipine besylate is the besylate salt of amlodipine, a long-acting calcium channel blocker. Amlodipine is a dihydropyridine calcium antagonist (calcium ion antagonist or slow-channel blocker) that inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. Amlodipine is a peripheral arterial vasodilator that acts directly on vascular smooth muscle to cause a reduction in peripheral vascular resistance and reduction in blood pressure [2]. Metoprolol is a beta1-selective (cardioselective) adrenergic receptor blocking agent. This preferential effect is not absolute, however, and at higher plasma concentrations, metoprolol also inhibits beta2-adrenoreceptors, chiefly located in the bronchial and vascular musculature. Metoprolol has no intrinsic sympathomimetic activity, and membrane-stabilizing activity is detectable only at plasma concentrations much greater than required for beta-blockade [3, 4].

In the fixed dose combination of amlodipine (calcium channel blocker) and metoprolol (cardioselective beta blocker); both the drugs have two different mechanisms and reduce blood pressure by acting on peripheral vascular resistance, stroke volume and heart rate. Advantages of this combination therapy effectively achieves target blood pressure, lower incidence of individual drug's side-effects, produces synergistic effects, increased patient compliance. Literature survey revealed few analytical methods are reported for analysis of both the drugs alone as well as in combination using UV spectrophotometry [5-10], HPLC [11-17], LC-MS [18] and HPTLC [19].

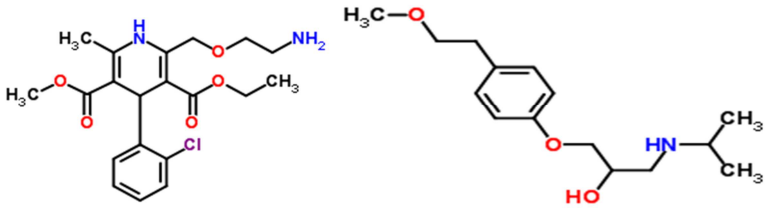


Figure-1: Chemical structures of Amlodipine, Metoprolol

To the best of our knowledge, there is no reported RP - HPLC method for simultaneous estimation of Amlodipine besylate and Metaprolol succinate in pharmaceutical formulations, previous to our work. Thus, efforts were made to develop fast, selective and sensitive analytical method for the estimation of Amlodipine besylate and Metaprolol succinate in their combined dosage form using reverse phase high performance liquid chromatographic method. Now the authors report a simple, reliable and reproducible RP-HPLC method which was duly validated by statistical parameters precision, accuracy and recovery. The method has been satisfactorily applied to the simultaneous estimation of Amlodipine besylate and Metaprolol succinate in bulk and pharmaceutical dosage forms. The developed method was validated as per ICH guidelines [20, 21].

MATERIALS AND METHODS

Bio Leo Labs pLtd. Hyderabad, Telangana, India was kind enough and supplied the reference standards of MET and AML for this research work. All the chemicals used throughout the research work were of analytical grade. Potassium dihydrogen orthophosphate was bought from Rankem Ltd., Mumbai, India. Acetonitrile (HPLC grade) and triethylamine (HPLC grade) purchased from Merck Pharmaceuticals Private Ltd., Mumbai, India. O-Phosphoric acid was also purchased from Merck Specialties Private Ltd., Mumbai, India. Commercial tablets of METOLOR-AM consist of MET (25 mg) and AML (5 mg) was purchased from local market manufactured by Cipla Limited, Mumbai, India.

Instruments and Chromatographic conditions

Chromatographic separations were attained by using Waters HPLC 2 2695 series consisting pump, Auto sampler, UV-Vis detector, Thermostat column compartment connected with Waters(alliance) Empower software (C_{18}, 150x4.6, 5µ particle size). 10 µL of sample was introduced into the HPLC system. The HPLC system data acquisition was performed with "Empower" software. Separations were executed on the reverse phase column comprising a mixture of 10 M Phosphate buffer (pH adjusted to 6.8±0.1 using phosphate buffer) and Acetonitrile in ratio of 65:35 v/v as mobile phase. The mobile phase was set at a flow rate of 1 mLminute^{-1} and eluent was monitored at 230 nm. In addition, an electronic balance (Shimadzu TX223L), digital pH meter (Systronics model 802), a sonicator (spectra lab, model UCB 40) were used in this present study.

Analytical methodology

Preparation of Reagents and Standards

Mobile phase

Precisely weighed 1.36gms of potassium dihydrogen phosphate and 0.3gms of dipotasssium hydrogen phosphate dissolved in 1000ml of distilled water, P^H was adjusted to 6.8±0.1 with dilute orthophosphoric acid.The above prepared buffer and acetonitrile were mixed in the proportion of 65:35 v/v. The mobile phase was then duly filtered through 0.45 µm nylon membrane vacuum filtration and duly degassed by sonication.

Preparation of Amlodipine and Metoprolol stock and standard solutions

5 mg of Amlodipine besylate and 25 mg Metaprolol succinate were weighed accurately and transferred in to 100 ml volumetric flasks. 30 ml of diluents were added and sonicated to dissolve the compound. This was made up to mark with buffer and acetonitrile in the ratio 65:35 v/v which yields 1000 µgml^{-1} (stock solution A). 10 ml of solution was pipetted out into 100ml volumetric flask and volume was made up to mark with diluents which gave 100 µgml^{-1} (stock solution B). The standard solution ranging from 2.5-15 mL and 25 -75mL were transferred into a series of 10 ml volumetric flasks to provide a final concentration range of amlodipine 2.5-15 µgml^{-1} and metoprolol 25-75 µgml^{-1}, and the contents of each flask was made up to the mark with diluents.

Preparation of Formulation Test Solution

Twenty tablets containing Amlodipine besylate and twenty tablets containing Metaprolol succinate were weighed and finely powered. An accurately weighed portion of the powder equivalent to 100 mg of Amlodipine besylate and Metaprolol succinate were transferred into 100 ml volumetric flasks. 10 ml of diluents were added and shaken for 20 minutes by manually and further sonicated for 10 minutes. This was diluted up to the mark with diluents. These solutions were centrifuged at 8000 rpm for 10 minutes. The supernatant solution was decanted into another test tube (. i.e. 1000 µgml^{-1}) 10 ml of supernatant solution were transferred into another 100 ml volumetric flask and made up to the mark with diluents (100 µgml^{-1}). Transfered 2.5-15 mL 12.5-75.0mL of solutions were transfer into another 10 ml volumetric flask and made up to the mark with diluents. The solution was filtered through 0.45 µm Nylon membrane filter paper. 20 µL of blank solution, placebo solution, three times of standard solutions were injected, disregarding peaks due to blank and placebo.

Assay procedure

The column was equilibrated for at least 30 minutes with mobile phase flowing through the system with a flow rate of 1.0 ml/min. Detector was set at a wavelength of 230 nm. Twelve sets of the drug solutions were prepared in diluents containing Amlodipine besylate and Metaprolol succinate at a concentration range of 2.5 - 15 µgml^{-1}and 12-75 µgml^{-1}. Then 20 µl of each standard and sample solution were injected for Six times separately. The retention time for Amlodipine besylate and Metaprolol succinate were found to be 2.769 and 4.116 min (Fig-2). The peak areas of the drug concentrations were calculated.

System suitability solution

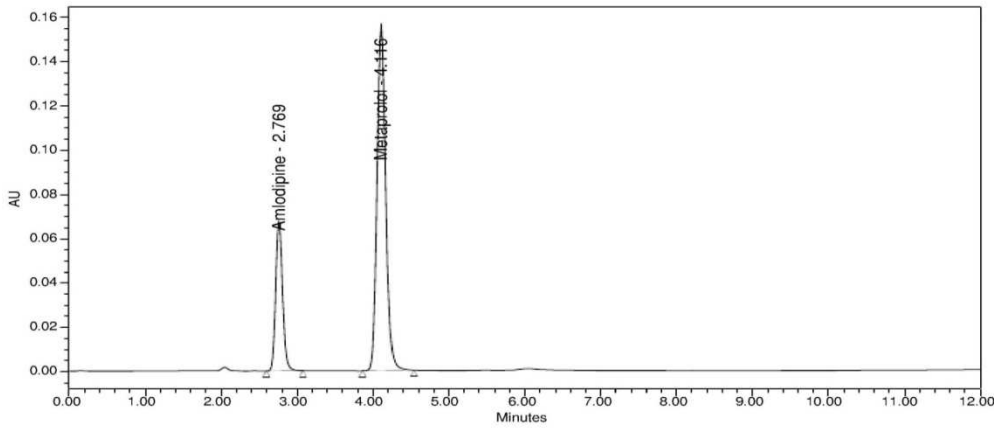
Used Amlodipine besylate and Metaprolol succinate standard working solution as system suitability solution.

Procedure

Equal volumes of blank were injected and twelve replicate injections of system suitability solutions in to column (Amlodipine besylate and Metaprolol succinate standard working solution). The chromatograms were recorded. Disregarded any peaks due to blank in the test solution. % RSD of twelve replicate injections of system was calculated (Amlodipine besylate and Metaprolol succinate standard working solution). Tailing factor and theoretical plates of the peak in the chromatogram obtained with 12th injection of system suitability solution (Amlodipine and Metaprolol standard working solution) were checked.

System suitability requirements from SST solution

a) Tailing factor : NMT 2.0
b) Theoretical Plates : NLT 2000
c) Resolution : NLT 2.0



	Peak Name	RT	Area	% Area	Height	USP Resolution	USP Plate Count	USP Tailing
1	Amlodipine	2.769	432480	25.72	67532		4398	1.13
2	Metaprolol	4.116	1249173	74.28	156062	7.06	6103	1.12

Figure-2: Sample Chromatogram of Amlodipine and Metaprolol

Linearity and Construction of Calibration Curve

Linearity of the peak area response was determined by taking measurements at twelve concentrations of working standard of Amlodipine besylate and Metaprolol succinate solutions in the range of 2.5-15 µg ml^{-1}and 12-75 µg ml^{-}

[1]. 20µL quantity of the solution was injected each time in to the column. The drug elutes were monitored at 230 nm at a column temperature of 30ºC and the corresponding chromatograms were recorded. The Linearity of the calibration curve was plotted between the mean peak areas versus respective Concentration in (Figs-3 & 4).

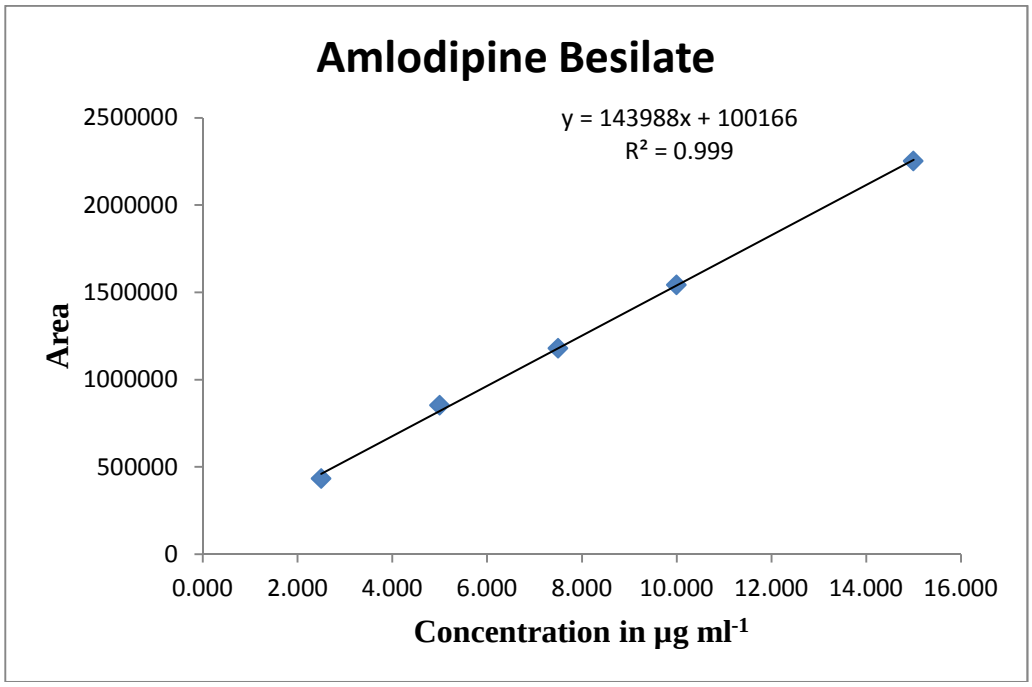


Figure-3: Linearity of Amlodipine

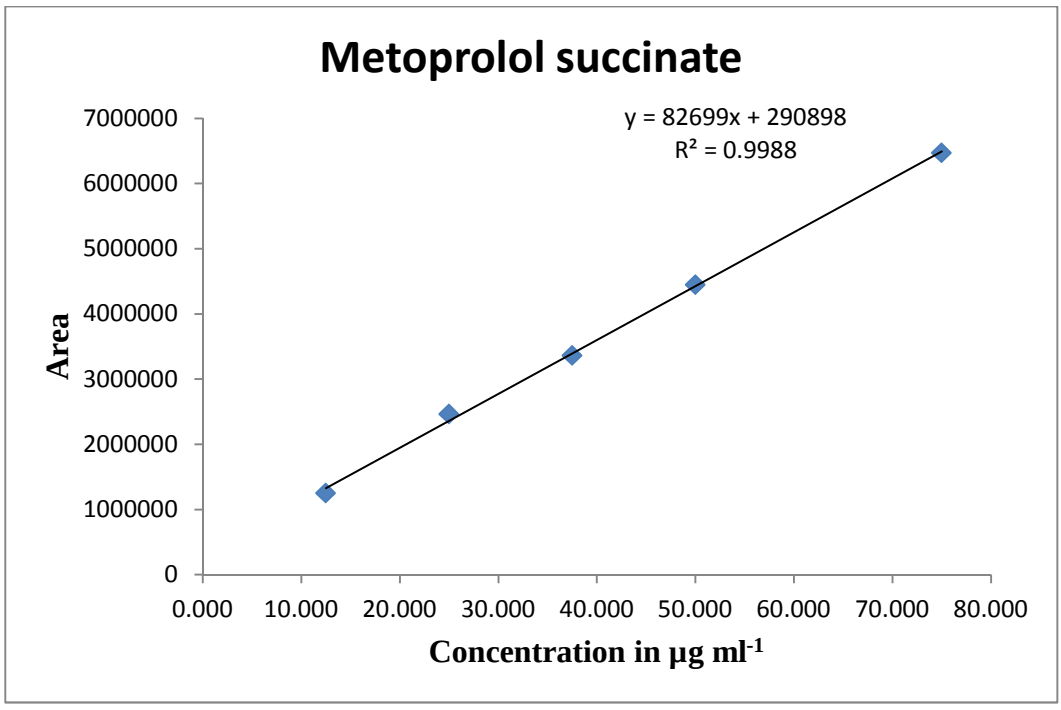


Figure-4: Linearity of Metoprolol

Table -1: Performance calculations, detection characteristics precision and accuracy of the proposed method for Amlodipine and Metaprolol

Parameter	HPLC Method for Amlodipine	HPLC Method for Metaprolol
Wavelength (nm)	230	230
Retention times (t) min	2.769	4.116
Linearity range (µg ml^{-1})	2.5-15	12-75
LOD (µg ml^{-1})	3.5	0.9311
LOQ (µg ml^{-1})	10.01	2.8214
Regression equation (y=bc+a)	---	---
Slope (b)	6.93803	1.2078
Intercept (a)	-0.68690	-3.46706
Correlation coefficient(r^2)	0.999	0.9998
Relative Standard deviation (%RSD)	0.91	0.60
Intermediate Precision (%RSD)	0.59	0.53

%RSD of Five independent determinations

Table-2: Results of linearity of sample

Amlodipine		Metoprolol	
Conc(µg)	Area	Conc(µg)	Area
2.500	432480	12.500	1249173
5.000	853222	25.000	2462309
7.500	1180551	37.500	3363935
10.000	1542249	50.000	4448908
15.000	2251855	75.000	6469979

Table -3: System precision and system suitability

S No	Amlodipine		Metoprolol	
	RT	Area	RT	Area
1	2.814	1581309	4.194	4544647
2	2.827	1599688	4.222	4594740
3	2.878	1598313	4.314	4603679
4	2.875	1576801	4.311	4602989
5	2.872	1565990	4.31	4602465
6	2.871	1564439	4.309	4602289
Avg	2.856	1581090	4.277	4591802
Std Dev	0.0280	15272.37	0.0539	23332.87
%RSD	0.982	0.966	1.261	0.508

Table -4: Method precision

S No	Amlodipine		Metoprolol	
	RT	Area	RT	Area
1	2.820	1601433	4.215	4634539
2	2.821	1600546	4.217	4634766
3	2.823	1589454	4.219	4645055
4	2.825	1585460	4.221	4689045
5	2.827	1599015	4.224	4701322
6	2.823	1595649	4.220	4700132
Avg	2.823167	1595260	4.219333	4667477
Std Dev	0.002563	6481.492	0.00314	32663.98
%RSD	0.09	0.41	0.07	0.70

Table-5: Ruggedness of Amlodipine Day 1 and Day 2

S No	Name	RT	Area
1	Injection-1	2.82	1601433
2	Injection-2	2.821	1600546
3	Injection-3	2.823	1589454
4	Injection-4	2.825	1585460
5	Injection-5	2.827	1599015
6	Injection-6	2.823	1595649
7	Injection-7	2.823	1576904
8	Injection-8	2.825	1569932
9	Injectoion-9	2.821	1570893
10	Injection-10	2.824	1565882
11	Injection-11	2.826	1569910
12	Injection-12	2.827	1570569
	AVG	2.82375	1582971
	STDEV	0.00234	13768.3
	%RSD	0.08	0.87

Table-6: Ruggedness of Metoprolol Day 1 and Day 2

S No	Name	RT	Area
1	Injection-1	4.215	4634539
2	Injection-2	4.217	4634766
3	Injection-3	4.219	4645055
4	Injection-4	4.221	4689045
5	Injection-5	4.224	4701322
6	Injection-6	4.22	4700132
7	Injection-7	4.22	4590012
8	Injection-8	4.223	4590434
9	Injectoion-9	4.221	4592167
10	Injection-10	4.227	4590344
11	Injection-11	4.229	4593454
12	Injection-12	4.231	4593019
	AVG	4.22225	4629524
	STDEV	0.004789	45357.71
	%RSD	0.11	0.98

Table-7: Robustness study of Amlodipine and Metoprolol

S.N	Peak Name	RT	Area	Height	% Area	USP Plate Count	USP Resolution	USP Tailing
1	AML Flow rate 1.1ml/min	3.398	1919989	248673	26.01	4457	-----	1.18
	MTP Flow rate 1.1ml/min	5.094	5460775	566876	73.99	6599	7.34	1.18
2	AML Flow rate 0.9ml/min	2.437	1341277	205925	25.82	3261	----	1.15
	MTP Flow rate 0.9ml/min	3.641	3852582	488660	74.18	5042	6.21	1.16
3	AMLTem-35˚C	2.833	1587672	224605	25.96	3773	-----	1.18
	MTPTem-35˚C	4.224	4527565	522537	74.04	5522	6.58	1.19
4	AMLTem-40˚C	2.817	1574133	224038	25.87	3773	-----	1.18
	MTPTem-40˚C	4.189	4511703	522392	74.13	5476	6.52	1.17

Table-8: Degradation study of Amlodipine and Metoprolol

S.N	Peak Name	RT	Area	Height	% Area	USP Plate Count	USP Resolution	USP Tailing
1	AML (Acid)	2.877	869127	128388	18.80	4142	1.53	1.15
	MTP (Acid)	4.311	2503868	296333	54.16	6185	5.49	1.16
2	AML (Base)	2.931	1163346	169437	21.16	4211	1.56	1.15
	MTP (Base)	4.380	3304255	384769	60.11	6103	5.44	1.16
3	AML (Oxi)	2.844	978877	141541	26.28	3906	1.47	1.16
	MTP (Oxi)	4.245	2688287	315752	72.16	5837	3.98	1.16
4	AML (UV)	2.851	889782	127996	26.36	3930	1.50	1.17
	MTP (UV)	4.279	2426841	281615	71.89	5786	4.03	1.16

Table-9: Assay Results of Amlodipine and Metoprolol

Drug	Amount present/tablet	Amount Found /tablet	% of Assay
Amlodipine	5	5.009	100.18
Metoprolol	25	24.56	98.23

Table-10: Accuracy data (Triplicate values at 50,100 &150 percent levels) of Amlodipine

S.No	Spike level	Peak area	Amount Added (µg/ml)	Amount Recovered (µg/ml)	%Recovery	Avg	% RSD
1	50%	807830	5.02	5.08	101.16	100.52	0.56
		803299	5.04	5.05	100.1		
		801210	5.02	5.04	100.31		
2	100%	1570934	10.01	98.75	98.65	98.83	0.16
		1571213	9.99	9.88	98.86		
		1570899	9.97	9.87	98.97		
3	150%	2368905	14.83	14.88	100.35	100.26	0.12
		2369145	14.87	14.89	100.12		
		2370154	14.85	14.9	100.31		

Table-11: Accuracy data (Triplicate values at 50,100 &150 percent levels) of Metoprolol

S.No	Spike level	Peak area	Amount Added (µg/ml)	Amount Recovered (µg/ml)	%Recovery	Avg	% RSD
1	50%	2325094	25.09	25.35	101.03	100.47	0.51
		2313218	25.21	25.22	100.02		
		2309892	25.09	25.18	100.35		
2	100%	4530865	50.02	49.38	98.72	98.91	0.17
		4532190	49.93	49.41	98.95		
		4531088	49.86	49.39	99.05		
3	150%	6700893	74.15	73.04	98.5	98.92	0.80
		6712809	74.33	73.16	98.43		
		6799032	74.23	74.11	99.84		

METHOD VALIDATION

The proposed method was validated as per ICH guidelines as follows. The UV absorption maximum for Amlodipine besylate and Metaprolol succinate was fixed at 230 nm. As the final detection was made by the UV absorption spectrum, each method was validated by linear fit curve.

Preparation of precision solution

10 ml of standard stock solution was taken in a 100 ml volumetric flask and was made up to the mark with diluents. The same procedure was repeated for remaining twelve more preparations. The percent of relative standard deviation was calculated for Amlodipine besylate and Metaprolol succinate and the results are presented in Table-4. The precision of the assays was also determined in terms of intra and inter-day variation in the peak areas for a set of drug solutions that were calculated in terms of coefficient variation. Acceptance Criteria: The %RSD of individual six samples preparations should not be more than 2.0%

Precision (Repeatability)

Precision was demonstrated by preparing twelve sample solutions as per the test method in a single batch. The precision of the method was ascertained separately from the peak area ratios obtained by actual determination of a fixed amount of drug. The percent of relative standard deviation was calculated for Amlodipine besylate and Metaprolol succinate and the results are presented in Tables- 5& 6. The precision of the assays was also determined in terms of intra and inter-day variation in the peak areas for a set of drug solutions and they were calculated in terms of coefficient variation.

Acceptance criteria: % RSD of individual % from twelve sample preparations should not be more than 2.0%.

System Suitability

System suitability of the proposed method is determined in terms of the parameters like tailing factor, theoretical plates and the standard deviation. The optical and system suitability parameters are tabulated in Table-3.

Linearity

The Linearity of the proposed method was checked over a concentration range 2.5 to 15µgml^{-1} and 12-75 µgml^{-1}. The regression concentration and areas are given in Table-2. The regression characters are given in Fig-3 & 4.

Accuracy

The accuracy of the test method was demonstrated preparing test samples with known quantities of Amlodipine besylate and Metaprolol succinate at the levels of 50%, 100%, and 150% concentration. The accuracy of the proposed method was determined using different technical grade samples of Amlodipine besylate and Metaprolol succinate within the linearity limits. The results are recorded in tables-10 &11.The results of recovery range

between 98.0% and 102.0%. The study proves that the method is accurate for the estimation of Amlodipine besylate and Metaprolol succinate.

Analysis of Formulation

The stability of the proposed method for the assay of formulations containing Amlodipine besylate and Metaprolol succinate was analyzed by the proposed and reference methods. The proposed method does not differ significantly in precision and accuracy from reference method.

Ruggedness

The ruggedness of test method was demonstrated by carrying out a precision study using six preparations. A single sample was studied by different analysts; the results of the intermediate precision (ruggedness) study are recorded in tables- 5 & 6.The mean % RSD for both method precision and intermediate precision is < 2.0.

Robustness

Robustness of the test method is the studied by variation of pH, variation of flow rate, and temperatures variation. The results are recorded in table-7. This results show that the method is robust.

Specificity and Selectivity

The specificity of the proposed method was determined by the complete separation of analyte and other components in the sample. The method does not permit detection of degradation product for furazolidone. Hence it can be concluded that the proposed HPLC method is accurate, precise, very fast and economical compared to those given in the literature. The results are recorded in Table-8.

Recovery Studies

Recovery studies were conducted by analyzing each formulation in the first instance for the active ingredient by the proposed methods. Known amounts of pure drug was then added to each of the previously analyzed formulations and the total amount of the Drug was once again determined by the proposed methods after bringing the active ingredient concentration within the linearity limits. The results are presented in Tables-10 &11.

LOD and LOQ

The proposed method shows that it has good sensitivity, the LOD values are found to be 3.5 and 0.9311, LOQ values are 10.01 and 2.82 respectively.

Solution Stability

The stability of the solutions under study was established by keeping the solution at room temperature for 48 hours. The results indicate no significant change in assay values. This indicates stability of drug in the solvent during analysis.

RESULTS AND DISCUSSION

The present study was aimed at developing a simple economical precise and accurate HPLC method for the analysis of Amlodipine besylate and Metaprolol succinate in bulk drug and in pharmaceutical. In order to achieve optimum separation of the component peaks, mixture of acetonitrile with water in different combinations were tested as mobile phase on a C_{18} stationary phase. The appropriate wavelength in UV region has been selected for the determination of active ingredient in the proposed method. This method was validated by linear fit curve and all other calculated parameters.

Parameters Fixation

Systematic study of the effects of various parameters was undertaken by varying one parameter at a time controlling all other parameters. The following studies were conducted for this purpose.

Mobile phase characteristics

In order to get sharp peaks and baseline separation of the components, a number of experiments were carried out by varying different components like percentage of organic phase in the mobile phase, total pH of the selected mobile phase and flow rate by changing one at a time and keeping all other parameters constant. The optimum conditions obtained were used in the procedure proposed.

Detection Characteristics

To test whether Amlodipine besylate and Metaprolol succinate had been linearly eluted from the column, different amounts of Amlodipine besylate and Metaprolol succinate were taken and analyzed by the above-mentioned

procedure. The peak area ratios of Amlodipine besylate and Metaprolol were calculated and the values are graphically represented in Fig-2. The optical and system suitability parameters are tabulated in Tables- 1 & 3. The retention time obtained for Amlodipine besylate and Metaprolol succinate were 2.769 and 4.116 min. A good linear relationship r^2 = 0.999 and r^2 = 0.9988 was observed indicating that the proposed method is linear over the range 2.5-15 µg/ml for AML and 25-75 µg/ml for MET. High recovery values between 99.83 to 102.06% obtained from the pharmaceutical dosages form by the proposed method indicates that the method is accurate. The deliberate changes in the method have not seriously affected the peak tailing, theoretical plates and the percent assay. This indicated the robustness of the method. When test solutions were analyzed by the proposed method to find out system precision and method precision low co-efficient of variation <1.0 was observed. The absence of additional peaks indicated non-interference of common excipients used in the tablets. The specificity of the HPLC method was determined by the complete separation with Amlodipine besylate and Metaprolol succinate. When it was subjected to forced degradation as per ICH guidelines using 0.1N HCL, 0.1N NaOH, heat degradation and oxidation. The method was specific. However the method does not permit detection of degradation product of Amlodipine and Metaprolol. The lowest value of LOD and LOQ were obtained in the proposed method using formula 3.3xstdev/slope for LOD and 10xstdev/slope for LOQ. The standard solution of the drug was stable up to 24 hrs as the difference in percent assay during the above period is within limit of the linear fit of the system which is illustrated graphically. Least square regression analysis was carried out for the slope, Intercepts and correlation coefficient. The results of assay are presented in Table -9.

CONCLUSION

The present proposed research study by the author describes the estimation of Amlodipine and Metaprolol available as combination tablet dosage forms and was carried out by utilizing RP-HPLC. The linearity of the proposed method was in the range of 2.5-15 µg/ml for AML and 25-75 µg/ml MET. The LOD and LOQ of AML were 3.5 µg/mL and 0.9311 µg/ml, and for the estimation of MET were 10.01 µg/ml and 2.8214 µg/ml respectively. The developed RP-HPLC method for the quantification of AML and MET were found to be simple, specific, highly sensitive, fast, economical, precise and extremely accurate with robustness. The developed method has several advantages like decorous linearity, less retention times and less solvent consumption which makes the method more economical than the existing methods in practice. Therefore this method can be recommended for the routine analysis of AML and MET in quality control and clinical laboratories.

Acknowledgements
The authors wish to thank Bio-Leo Labs Ltd., Hyderabad for supplying the samples of Metoprolol and amlodipine within the required time so as to enable us to complete this research paper quickly. We also highly thankful to department of chemistry Sri Venkateswara University for providing the necessary lab facilities to carry out this research work successfully.

REFERENCES

[1] A. Domenic, *Sica Drugs* 62 (**2002**) 443–462.
[2] Norvasc, Online Drug Description and Clinical Pharmacology of amlodipine, The Internet Drug Index, RxList Inc., **2008**. Website: http://www.rxlist.com/cgi/generic/amlod2.htm.
[3] TOPROL-XL®, Online Clinical Pharmacology of metoprolol, The Internet DrugIndex, RxList Inc., 2008. website: ttp://www.rxlist.com/cgi/generic/metopxl.htm.
[4] J. Hainer, *J. Sugg, Vasc. Health Risk Manag*. 3 (**2007**) 279.
[5] K.D. Tripathi, Essential of medical Pharmacology, 6th edition, New Delhi, **2008**; 539.
[6] A.S. Jadhav, K.N. Tarkase, A.P. Deshpande, *Der Pharmacia Letter*. 4(**2012**) 763-767.
[7] S. D. Sawant, M. R. Ghante, A. S. Deshpande, B. Shah, *International Journal of Chemical and Analytical Science*. 1(**2010**) 217-218.
[8] M. Badulescu, D. Balalau, *Farmacia*. 6(**2008**) 363- 370.
[9] K. Sureshkumar, R. Ravikumar, A. Rajasekaran, V. Ravichandran, *Digest J Nano Bio* 5(**2010**) 173-176.
[10] G. Ragno, A. Garofalo, C. Vetuschi, *J Pharm Biomed Anal*. 27 (**2002**) 19-24.
[11] P.N. Dhabale, S.R. Bhagade, *J Chem Pharm Res*. 3 (**2011**) 650-656.
[12] M. D. Phale, P. D. Hamrapurkar, *Asian J Research Chem*. 2(**2009**) 119-122.
[13] M. Aqil, A. Ali, A. Ahad, Y. Sultana, A.K. Najmi, N. Saha, *Acta Chromatogr*, 19 (**2007**) 130-140.
[14] H. Salem, O.M. Abdallah, *Am J Appl Sci*. 4(**2007**) 709.
[15] P. D. Varma, A. Lakshmana Rao, S.C. Dinda, *Int J Res Pharm Chem*. 2 (**2011**) 876-884.
[16] M.M. Prasada Rao, S.A. Rahaman, Y.R. Prasad, P.G. Reddy, *International Journal of Pharmaceutical Research and Development.* 2 (**2010**) 69-76.
[17] P.N. Pai, K.R. Shenoy, J. Pandey, *Indian J Pharma Sci*. 67 (**2005**) 608-610.

[18] J. Bagyalakshmi, S. M. Philip, T.K. Ravi, *Der Pharma Chemica*. 3 (**2011**) 140-145.
[19] J. Bhatt, S. Singh, G. Subbaiah, B. Shah, S. Kambli, S. Ameta, *J Biomed Chromatogr Sci Appl*. 21 (**2007**) 169-175.
[20] S.N. Meyyanathan, B Suresh, *J Chromatogr Sci*. 43 (**2005**) 73-5.
[21] ICH Guidelines Q2(R1). Validation of Analytical Procedures: Methodology International Conference on Harmonization, Geneva, **2005**.