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Original Article

Method Development and Validation of Antihypertensive Drugs Using HPLC Technique

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ABSTRACT

Aim: A validated RP-HPLC method was established for the quantification of Amlodipine and Metoprolol succinate according to ICH guidelines. **Materials and Methods:** This method utilized a Phenomenex (US) C_{18} column with dimensions of 250X4.6 mm I.D and a particle size of 5 µm. Employing an isocratic elution technique, the mobile phase comprised pH 3.0 phosphate buffer solution and acetonitrile. Detection occurred at a wavelength of 215 nm and a flow rate of 1 mL/min was chosen to ensure optimal resolution for Amlodipine and Metoprolol succinate. **Results and Discussion:** Retention times were noted at 3.137 min and 5.672 min for Amlodipine and Metoprolol succinate, respectively. High correlation coefficients were achieved (0.9995 for Amlodipine and 0.9996 for Metoprolol succinate). The method exhibited precision, with low relative standard deviations of 0.357% for Amlodipine and 0.077% for Metoprolol succinate. In summary, the RP-HPLC method developed was specific, linear, precise and robust. **Conclusion:** The devised method was validated in terms of accuracy, precision, linearity, specificity, robustness and ruggedness. All formulations' sample recoveries were in good accordance with the claims made on their labels

Keywords: Amlodipine, Metoprolol succinate, Retention time, Correlation coefficient, RP-HPLC.

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INTRODUCTION

Amlodipine besylate is indeed a long-acting calcium channel blocker. It works by inhibiting the influx of calcium ions into vascular smooth muscle and cardiac muscle cells, leading to vasodilation (relaxation of blood vessels) and a reduction in myocardial contractility. This makes it effective in treating hypertension (high blood pressure) and angina (chest pain) caused by coronary artery disease.[1] Metoprolol succinate is a beta-adrenergic blocking agent, specifically a β1-receptor blocker. It works by blocking the effects of the hormone adrenaline on the heart, reducing heart rate and blood pressure. This makes it useful in the treatment of hypertension, angina and certain cardiac arrhythmias. Both medications are commonly used in the management of cardiovascular conditions, but they belong to different classes of drugs with distinct mechanisms of action. Sometimes, they may be prescribed together to provide a more comprehensive treatment approach for certain cardiovascular conditions.[2,3]

The manufacturing industry heavily relies on both qualitative and quantitative analyses to guarantee that raw materials adhere to specified standards and to assess the quality of the final product. The method devised for these analyses underwent validation according to the guidelines set by the International Council for Harmonisation (ICH). The validation process included experiments to assess accuracy, precision, specificity, Limit of Detection (LOD), Limit of Quantitation (LOQ), linearity, range, robustness and ruggedness, all conducted using Reverse Phase High-Performance Liquid Chromatography (RP-HPLC). RP-HPLC, a versatile separation technique, was chosen for its broad applicability, outstanding resolution capabilities, rapidity and ability to detect at the nano-molecular level. This method ensures the reliability and accuracy of analytical results, supporting quality control and assurance in the manufacturing sector.[4,5]

MATERIALS AND METHODS

Chemical required

o-phosphoric acid, acetonitrile, mono basic sodium phosphate and distilled water.

Active ingredients

Amlodipine besylate, Metoprolol succinate.

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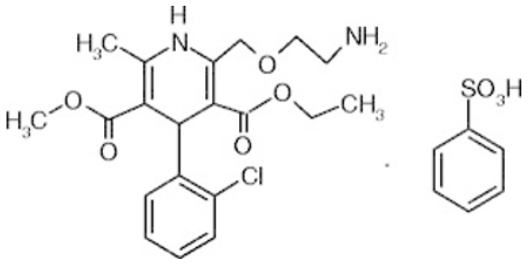
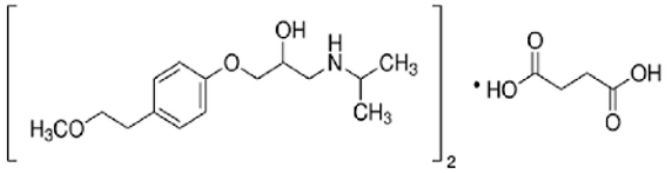


Amlodipine besylate



Metoprolol succinate

Instruments used

Sartorius balance, Mettler-Toledo balance, pH meter, Ultra sonicator, Water purifier, HPLC, HPLC Detector, PDA Detector.

Selection of wavelength

Preparation of Solution for UV Scanning

100 mg of Metoprolol succinate was dissolved in a 100 mL volumetric flask and made up to the volume with water. Similarly, 50 mg of Amlodipine besylate was dissolved in a separate 100 mL volumetric flask and made up to the volume. Subsequently, both solutions were scanned over a range of 400-200 nm using a medium scan speed.

Preparation of standard and sample solution

Preparation of working standard solution

Stock 1 weigh accurately 70.0 mg of Amlodipine besylate WS (equivalent to Amlodipine 25 mg) and 475 mg Metoprolol succinate WS in 100 mL volumetric flask, add 25 mL of water dissolve and makeup with same solvent up to the mark. Stock 2 further dilute 5 mL to 50 mL with water. (Amlodipine -50 ppm and Metoprolol-475 ppm).

Preparation of Sample solution

Crush one tablet containing 5 mg of Amlodipine and 47.5 mg of Metoprolol succinate and transfer it into a 100 mL volumetric flask. Introduce 25 mL of water into the flask, followed by sonication and shaking for 30 min. Complete the volume to 100 mL with water. Filter the solution through a 0.45 µ membrane filter. The resulting concentrations are 50 ppm for Amlodipine and 475 ppm for Metoprolol.

Preparation of Mobile Phase

Preparation of pH 3.0 Phosphate buffer

Combine 50 mL of 1 M monobasic sodium phosphate with 8.0 mL of 1 M phosphoric acid and then dilute the mixture with water to a total volume of 1000 mL. If needed, make adjustments using 1 M monobasic sodium phosphate or 1M phosphoric acid to achieve a pH of 3.0.

Preparation of 1 M Monobasic sodium phosphate

Solution of 1 M prepared by diluting 119.98 g of monobasic sodium phosphate to 1000 mL with water.

Preparation of 1M Phosphoric acid

Solution of 1 M prepared by diluting 98.0 g or 56 mL solution of phosphoric acid in 100 mL with water.

Preparation of mobile phase composition (%v/v)

Prepared Phosphate buffer of pH 3.0 (750 mL) and ACN (250 mL) were kept in channel B and channel A of HPLC system, respectively.

Validation of developed method

ICH guidelines were followed in the validation of the devised approach. All parameters in the mobile phase were set up and prepared using the previously optimal approach (Tables 1 and 2).[7-12]

Specificity

Specificity refers to the method's capability to measure the analyte accurately even in the presence of other substances or ingredients. Placebo preparation:

- Lactose
- HPMC K-100 M
- Sodium CMC
- Poly vinyl pyrrolidine
- Iso propyl alcohol
- Purified tale
- Magnesium stearate.

Determination

Showing that the procedure is not affected by presence of Inactive materials or excipient by doing the spiking of the drug substance and demonstrating the assay result is unaffected establish the specificity.

Procedure

Preparation of placebo solution

Take 1 tablet content of individual inactive material (367.5 mg) in to 100 mL volumetric flask add 25 mL of water and sonicate shake it for 30 min. Finally, makeup to the mark with water. Filter through 0.45 µ filter and inject.

Preparation of standard solution of Amlodipine and Metoprolol

Solution was prepared as given in procedure for preparation of standard solution of Amlodipine and Metoprolol succinate under system suitability.

Preparation of Sample solution

Take 1 tablet, equivalent to 5 mg Amlodipine and 47.5 mg of Metoprolol succinate crush and take it in 100 mL volumetric flask. Add 25 mL of water and sonicate take in for 30 min. Finally, make up to the mark with water. Solution was filtered through 0.45 µ membrane filter. (Amlodipine -50 ppm and Metoprolol succinate-475 ppm).

Linearity and range

Separately weigh 80%, 90%, 100%, 110%, 120% and 140% of regular assay concentration and perform the assay. The responses are plotted a graph against concentrations. Correlation co-efficient calculated (Table 3).

Chromatograms were run for the above linearity solution.

Recovery Accuracy

Procedure

The accuracy of the method is determined by recovery experiments. The recovery is permed by adding Amlodipine besylate and Metoprolol succinate WS to Placebo (Amlovas tablets excipients mixture) in the range of 80% 120%. Mix well and performed the assay of the mixed homogenous sample, in triplicate, in the specified assay range. Find the percentage-recovery of the analyte by comparing with the regular assay value of the sample. The percentage recovery should be 100.42%, The analysis precision is measured at 100% concentration levels expressed as %RSD.

Precision

Repeatability

Repeatability was evaluated by conducting a minimum of six determinations at 100% of the test concentration.

Intermediate precision/ruggedness

Regular assay can be performed with a homogeneous sample by changing the following few conditions in duplicate. 1. Different Analysts, 2. Different Days.

Procedure: The homogeneous sample given to another two different analysts and perform the assay without changing the prescribed standard procedure in the same laboratory. Recorded the assay results. Perform the assay for the same sample on next day in duplicate and record the assay results. The relative standard deviation of all the results should not be more than 2.0%. The concentrations of 100% Test solution.

Preparation of sample solution

solution was prepared as given in procedure of preparation of sample solution.

Robustness

A technique's robustness was evaluated by calculating how long it could withstand tiny, intentional changes in the method parameters. This test indicates how reliable the procedure would be under typical operating conditions (Table 4).

Table 1: Getting started on method development.[5,6]

Goal	Content
Separation time in HPLC	A time frame of less than 5-10 min is considered desirable for routine procedures.
Quantitative detection	Not exceeding 2% for assays and not surpassing 5% for less demanding analyses. Less than 15% for trace analyses.
HPLC pressure	Desirably, pressures should be below 150 bars; however, a typically essential value, especially with a new column, is around 200 bars.
Chromatographic peak height	Desirable signal-to-noise ratios are achieved when peaks are narrow.
Solvents used	It is advantageous to minimize the use of mobile phase per run.

Table 2: Chromatographic condition of optimized method.

Parameters	Description
Column	C_{18} column, 5µ (250x4.6).
Mobile phase	Phosphate buffer (20 mM); acetonitrile (75:25). pH was adjusted to 3.0 with orthophosphoric acid.
Injection volume	15 µL
Flow rate	1.0 mL/min
Detector wavelength	UV at 215 nm
Temperature	Ambient

RESULTS AND DISCUSSION

The present investigation was to develop a new method and validation of Amlodipine and Metoprolol succinate in pharmaceutical dosage form by RP-HPLC (Table 5 and Figure 1).

UV wavelength

UV spectrum was recorded for Amlodipine and Metoprolol succinate individually the absorbance maximum of Amlodipine and Metoprolol succinate was found to be 215 nm at point of Isosbestic. Hence suitable wavelength for detection of Metoprolol succinate and Amlodipine was 215 nm.

From the above experiment it was found that Amlodipine and Metoprolol succinate can effectively be analysed by the RP-HPLC method with Phosphate Buffer (3.0 pH): Acetonitrile (75:25) as the mobile phase at a flow rate of 1.0 mL/min and detection wavelength of 215 nm. The retention time of the drugs was 3.137 and 5.672 min.

Method validation

System suitability and specificity

Table 3: Preparation of solution for linearity.

Sl. No.	Concentration (%)	Weight taken to the sample solution (mg)	Dilute up to	Dilute with
1	80%	333.2	100 mL	Diluent
2	90%	374.85	100 mL	Diluent
3	100%	416.5	100 mL	Diluent
4	110%	458.15	100 mL	Diluent
5	120%	499.8	100 mL	Diluent
6	130%	583.1	100 mL	Diluent

Table 4: Robustness Parameters.

Method Parameter	Method Details	Changes to the method
Injection volume	15 µL	a.13 µL b.17 µL
Flow rate	1 mL/min	a.0.8 mL/min b.1.2 mL/min

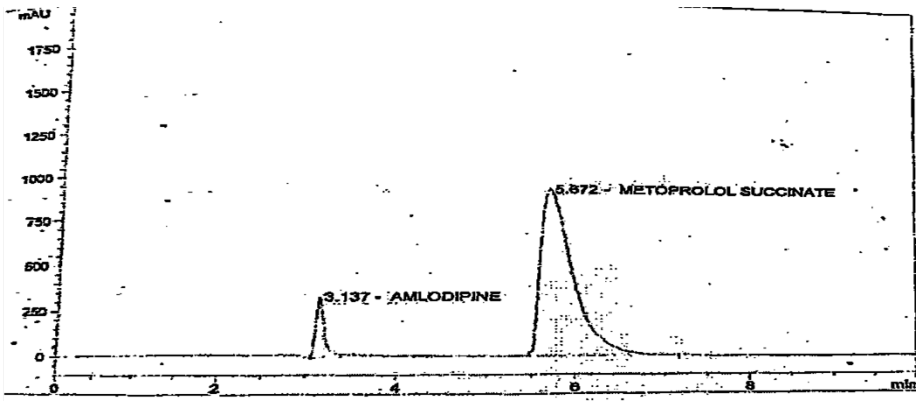


Figure 1: HPLC Chromatogram optimized trial.

RESULTS

In system suitability R.S.D for amlodipine was 1.53% for metoprolol succinate was 0.03%. For amlodipine and metoprolol mean RT and Tailing year was found to be 3.133 min and 1.222 and 5.663 min and 1.160 respectively. In specificity there was no interference by placebo, no impurities (Table 6, Figures 2, 3, 4 and 5).

Linearity

Result: The linearity of proposed method found to be R^2=0.9995 and R^2= 0.9996 for amlodipine and metoprolol succinate (Tables 7 nd 8 and Figures 6 and 7).

Accuracy

Result: The % recovery of amlodipine was 102.2, 102.0, and 101.60, at 80%, 100%, and 120% level where as for metoprolol succinate was 100.21, 100.08, and 100.04 at 80%, 100% and 120% levels (Tables 9, 10 and Figures 8, 9).

Precision

Intermediate precision

Result: S.D of amlodipine and metoprolol succinate was 1.214 and 4.015 respectively whereas R.S.D for amlodipine and metoprolol succinate was 0.060% and 0.018% respectively (Tables 11-14 and Figures 10, 11).

Table 5: Chromatogram optimized trial for amlodipine and metoprolol succinate.

Sample name: Amlovas-M.
Sample ID: Standard.
Injection volume: 15 µL.
Method information: assay of amlodipine and metoprolol succinate.

ID. No.	Name of API	RT (min)	Area
1	Amlodipine	3.137	2010.143
2	Metoprolol succinate	5.672	23030.607

Table 6: Data for system suitability of amlodipine and metoprolol succinate.

System suitability parameter	RT (min)	AUC	Tailing factor
Rep-1	3.137	2010.143	1.221
Rep-2	3.138	2018.907	1.217
Rep-3	3.134	2035.632	1.229
Rep-4	3.134	2081.999	1.221
Rep-5	3.132	2018.303	1.217
Rep-6	3.128	2028.835	1.229
Mean	3.133	2042.814	1.222
S. D	0.0015	31.304	0.0061
%R.S. D	0.0582	1.53	0.499
Rep-1	5.672	23030.607	1.158
Rep-2	5.672	23020.580	1.159
Rep-3	5.661	23038.896	1.164
Rep-4	5.661	23037.809	1.158
Rep-5	5.660	23033.480	1.159
Rep-6	5.656	23038.619	1.164
Mean	5.663	23033.332	1.160
S. D	0.0053	7.057	0.0026
%R.S. D	0.103	0.03	0.226

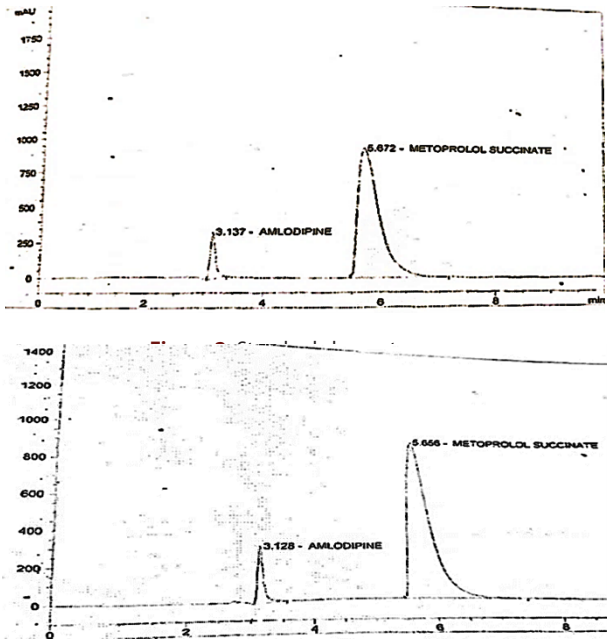


Figure 3: Blank chromatogram.

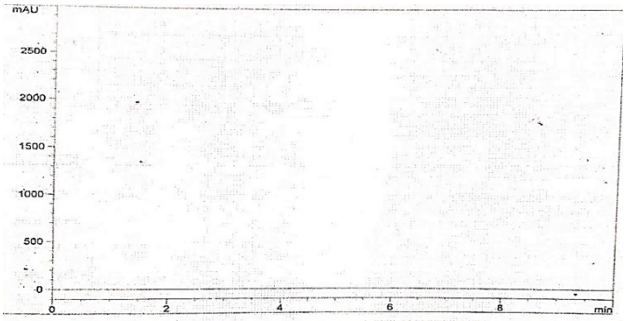


Figure 4: Sample chromatogram..

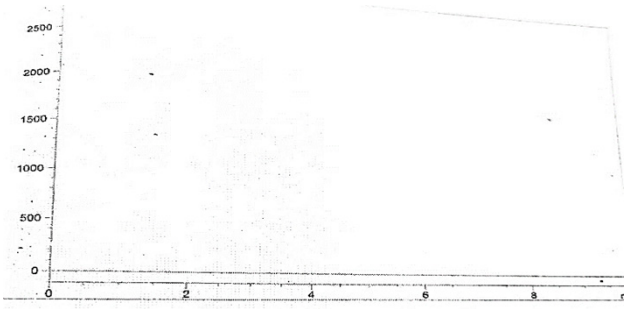


Figure 5: Placebo chromatogram.

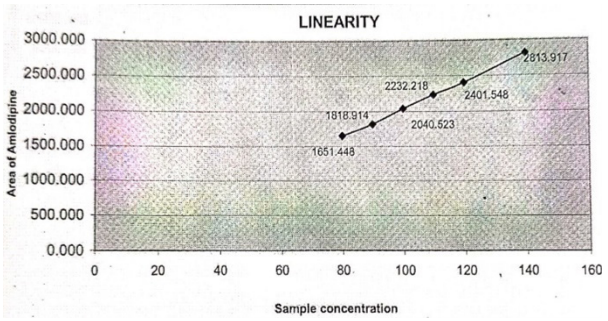


Figure 6: Linearity of amlodipine.

Table 7: **Data for linearity of amlodipine.**

Sl. No.	Concentration	AUC
1	80 µg/mL	1651.448
2	90 µg/mL	1818.914
3	100 µg/mL	2040.523
4	120 µg/mL	2232.218
5	130 µg/mL	2401.548
6	140 µg/mL	2813.917
Correlation coefficient		0.9995

Table 8: **Data for linearity of metoprolol.**

Sl. No.	Concentration	AUC
1	80 µg/mL	18576.908
2	90 µg/mL	20754.811
3	100 µg/mL	23074.584
4	120 µg/mL	25397.213
5	130 µg/mL	27648.693
6	140 µg/mL	32219.318
Correlation coefficient		0.9996

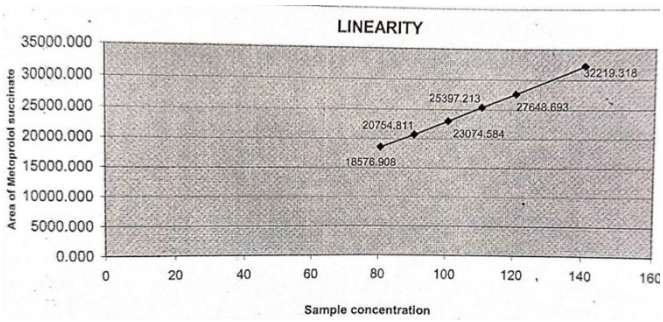


Figure 7: Linearity of metoprolol.

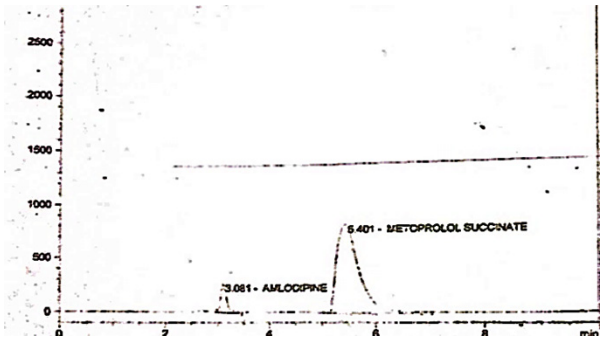


Figure 8: 100% level of accuracy.

Table 9: Data for recover of amlodipine.

Level	Sl. No.	Peak area	% recovery	Mean	S.D	% RSD
80%	1	1642854	101.90	102.2	2.049	0.125
	2	1638.843	102.50			
	3	1640.123	102.20			
100%	1	2032.682	101.9	102.2	3.017	0.148
	2	2036.323	102.0			
	3	2038.669	102.0			
120%	1	2411.330	101.35	101.60	1.323	0.055
	2	2140.130	101.55			
	3	2408.687	101.90			

Table 10: Data for recovery of metoprolol succinate.

Level	Sl. No.	Peak area	% recovery	Mean	S.D	% RSD
80%	1	18597.869	100.08	100.21	16.267	0.088
	2	18570.289	100.23			
	3	18569.135	100.32			
100%	1	23065.563	100.02	100.08	7.939	0.034
	2	23079.537	100.10			
	3	23079.080	100.12			
120%	1	27620.193	100.01	100.04	13.629	0.049
	2	27638.152	100.03			
	3	27646.930	100.08			

Table 11: Repeatability.

Sl. No.	Amlodipine		Metoprolol succinate	
	AUC	Amount (%)	AUC	Amount (%)
1	2030.495	100.42	23051.14	100.97
2	2023.638	100.29	23074.469	100.45
3	2029.326	101.44	23048.736	99.57
4	2042.096	101.49	23057.074	99.51
5	2024.702	100.32	23083.500	100.33
6	2020.387	98.65	23042.283	98.67
Avg.	2028.441	100.10	23059.535	99.92
S.D	7.660	0.710	16.050	0.821
R.S. D	0.378	0.710	0.070	0.822

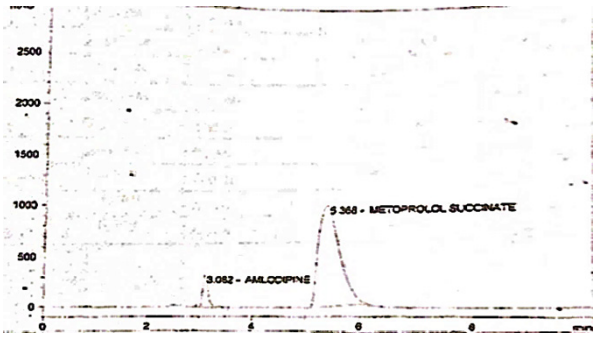


Figure 9: 120% level of accuracy.

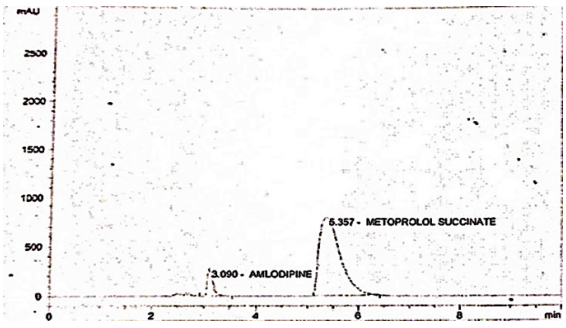


Figure 10: Intermediate precision analyst-1/day-1.

Table 12: Data for assay by Analyst I/Day I.

Sl. No.	Area of amlodipine	Area of metoprolol succinate
1	2023.843	23055.842
2	2025.027	23067.625
3	2027.509	23057.311
Avg.	2025.460	23060.259
SD	1.871	6.421
%RSD	0.092	0.028

Table 13: Data for assay by analyst II/day II.

Sl. No.	Area of amlodipine	Area of metoprolol succinate
1	2023.843	23055.842
2	2025.027	23067.625
3	2027.509	23057.311
Avg.	2025.460	23060.259
SD	1.871	6.421
%RSD	0.092	0.028

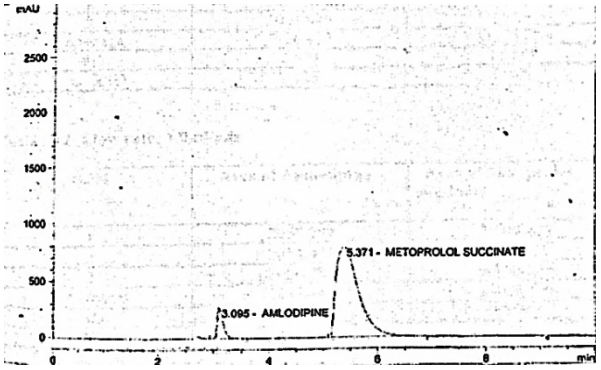


Figure 11: Intermediate precision analyst-2/day-2.

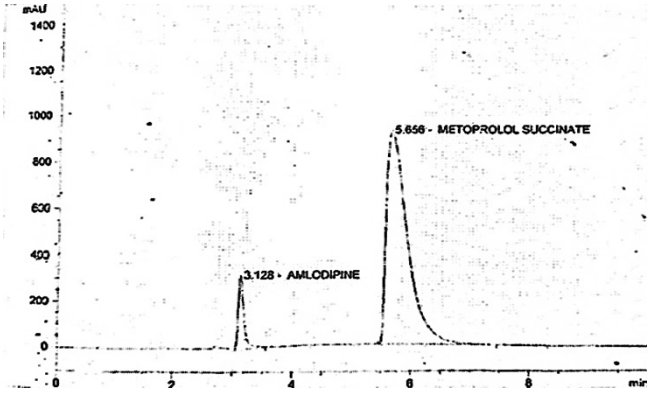


Figure 12: Chromatogram at flow rate 1.0 mL.

Table 14: Ruggedness test result.

Sl. No.	Test	%RSD Amlodipine	%RSD Metoprolol	Average amlodipine	Average Metoprolol
1	Analyst I/ day I	0.092	0.028	0.060	0.018
2	Analyst II/ day II	0.027	0.007		

Table 15: Flow rate 0.8 mL/min.

Sl. No.	Area of amlodipine	Area of metoprolol succinate
1	2028.566	23063.557
2	2028.221	23097.070
3	2024.023	23064.990
Avg.	2026.937	23075.206
SD	2.529	18.949
%RSD	0.125	0.082

Table 16: Flow rate 1.0 mL/min.

Sl. No.	Area of amlodipine	Area of metoprolol succinate
1	2057.099	23059.111
2	2054.441	23062.400
3	2060.780	23078.258
Avg.	2057.440	23066.590
SD	3.183	10.238
%RSD	0.155	0.044

Table 17: Flow rate 1.2 mL/min.

Sl. No.	Area of amlodipine	Area of metoprolol succinate
1	2065.577	23089.502
2	2064.475	23099.389
3	2065.229	23094.494
Avg.	2065.094	23094.462
SD	0.563	4.944
%RSD	0.027	0.021

Table 18: Robustness test results.

Sl. No.	Test	%RSD	%RSD	Average	Average
		Amlodipine	Metoprolol	Amlodipine	Metoprolol
1	8.0 mL	0.125	0.082	0.102	0.049
2	1.0 mL	0.155	0.044		
3	1.2 mL	0.027	0.021		

Robustness

Result: RSD with decrease injection volume for amlodipine was 0.086% and for metoprolol succinate was 0.031%. RSD with increase injection volume for amlodipine was 0.223% and for metoprolol succinate was 0.070% (Tables 15-18 and Figure 12).

CONCLUSION

It was discovered that the suggested HPLC approach for the simultaneous estimation of amlodipine and metoprolol succinate in pharmaceutical dosage form was straightforward, specific, accurate, fast and affordable. The results will be statistically validated in accordance with ICH requirements. The devised method was validated in terms of accuracy, precision, linearity, specificity, robustness and ruggedness. All formulations' sample recoveries were in good accordance with the claims made on their labels. For the ordinary analysis of simultaneous estimates, it can therefore be used. Therefore, the RP-HPLC method can be used for dissolving investigations as well as quality control of formulations and raw materials. Studies on bioequivalence in plasma can make use of it.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

HPLC: High performance liquid chromatography; **RSD:** Relative standard deviation; **SD:** Standard deviation; **AUC:** Area under the curve; **RT:** Retention time; **ICH:** International council of harmonization; **ACN:** Acetonitrile.

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