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Eco-Friendly Stability-Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Metoprolol Succinate, Amlodipine Besylate, and Chlorthalidone

Jignesh Jasmatbhai Kanzariya[1], Hiren Kadikar[2], Mona Kaushal[3]
[1]Phd Scholar, Swarrnim Startup & Innovation University, Gandhinagar.
[2]Registrar, Swarrnim Startup & Innovation University, Gandhinagar.
[3]Principal, Smt.S.M. Shah Pharmacy College, Ahmedabad.

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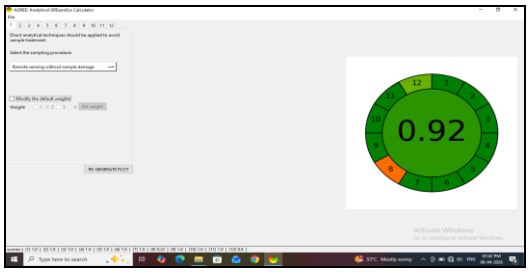
Metorolol Succinate, Amlodipine Besylate, Chlorthalidone, Method validation, Stability Study, HPLC Method, Greenness assessment.

ABSTRACT:
Background: Metoprolol Succinate, Amlodipine Besylate & Chlorthalodone are three medications that diminish arterial pressure. The current study comprises an evaluation of the proposed methodologies greenness regarding the HPLC method used to govern the medication mixture regardless of dose forms a novel stability suggesting HPLC methods environmental effect was evaluated using the greenness metrics. Stress condition comprising acidic, alkaline, oxidative, and thermal degradation were applied for three drugs of the medications. **Results:** The HPLC method employing a C_{18} column with a gradient approach, the HPLC chromatography was carried out. The mobile phase consisted of 0.02M Potassium Dihydrogen Phosphate: Acetonitrile (45:55 % v/v), with the stationary phase being the Hibar ODS C18 column. The HPLC method use UV detection at 242 nm with chromatographic purification spanning LINEARITY of 25-125 µg/ml for Metoprolol Succinate, 2.5-12.5 µg/ml for Amlodipine Besylate, and 6.25-31.25 µg/ml for Chlorthalidone, correspondingly. The procedure is accurate and precise, as demonstrated by an outcome that %RSD inside the permissible range. Additionally, various stressors were introduced to the medications. The approach's green credentials with respect to solvent utilization, chemical substances, expenditure of energy, and waste formation have been verified by the greenness data collected during the evaluation. No chromatographic or spectrum impediments caused by formulation additives have been observed. **Conclusion:** Metorolol Succinate, Amlodipine Besylate, and Chlorthalidone could be measured simultaneously using the devised HPLC method, which was simple, quick, sensitive, accurate, precise, linear, and stability indicating. The proposed approach showed ecological friendliness, robustness, sensitivity, and ease of use. As a result, the devised method could be applied to the regular quality checking of tablets and bulk medications.



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INTRODUCTION:

Metoprolol Succinate (MET) is a beta-blocker, Amlodipine Besylate (AML) is a Calcium channel blocker, and Chlorthalidone (CHLOR) is a Diuretic, so that use to treat several or resistant high blood pressure and stable angina. It works by lowering heart rate, relaxing blood vessels and reducing blood volume to efficiently manage cardiovascular health.

When combine they improve the heart's rate ability to circulate blood efficiently and even more smoothly. Metoprolol Succinate (MET), chemical name is (*RS*)-1-(Isopropylamino)-3-[4-(2-methoxyethyl)phenoxy] propan-2-ol(Fig. 1), Metoprolol Succinate comprises a white color powder that dissolves in methanol, water, ethanol (95%), and isopropyl alcohol. This drug to mechanism of slows heart rate and reduces the heart's workload.

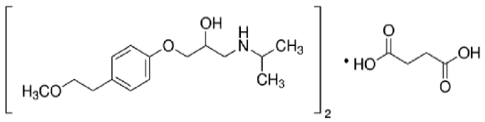

Fig.1 Structure of Metoprolol Succinate

Amlodipine Besylate (AML), chemical name is (RS)-3-ethyl-5-methy-l-2-(2-amino ethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3, 5 pyridine dicarboxylate benzene sulfonate (Fig. 2). Amlodipine Besylate is a white to yellowish white color powder, which extremely in soluble in methanol, water, slightly soluble in 2-propanol. Long acting calcium channel blocker, this is used as an antihypertensive agent.

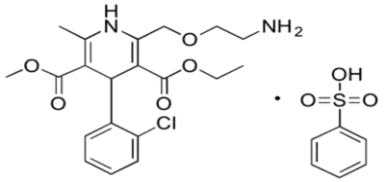

Fig. 2 Structure Amlodipine Besylate

Chlorthalidone (CHL), chemical name is 2-chloro-5-(1-hydroxyl-3-oxo-2, 3-dihydro-1Hisoindol-1-yl) benzene-1-sulfonamide (Fig. 3). Chlorthalidone is a yellow or white color, which is Soluble in methanol, slightly soluble in ethanol 95%, practically insoluble in water, in ether & in chloroform. Long-acting diuretic used to treat hypertension (high blood pressure) and edema (fluid retention).

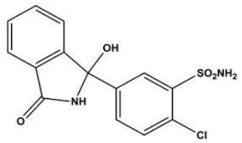

Fig. 3 Structure chlorthalidone

Three areas that are gaining popularity in the chemical community are putting green chemistry in to practice and emphasizing it in the laboratory. Chemists worldwide are working to replace traditional methods and reactions with more energy-efficient, sustainable ones. Green chemistry can take many different forms, like actively managing trash or rethinking research using more eco-logically friendly chemicals.

Numerous ways have been developed to assess the effectiveness of green chemical techniques against traditional ones. This strategy can be recalled by using the term significance and is based on the twelve guiding principles of green analytical chemistry.

Analytical chemistry is one field that is attempting to integrate concepts of green chemistry into tools and analytical processes. One of the main issues in analytical chemistry is developing sample preparation procedures that are more robust and efficient. According to the principle, the most practical way to reduce waste is to introduce the sample

with little to no preparation.

When sample preparation is necessary, the second, fifth, seventh, ninth, tenth, eleventh, and twelve principles may and ought to be applied. To reduce the quantity of energy required, solvent use, waste output, and employee contact, sample preparation methods should be enhanced as much as possible. One area of chemical analysis that GAC is particularly interested in is chromatographic techniques, which are employed to separate the constituents of complex mixtures in various matrices.

Following a comprehensive literature study, it was found that various approaches to the assessment of Metoprolol Succinate, Amlodipine Besylate and chlorthalidone using various analytical UV, HPLC, techniques, and stability indicating method for Metoprolol Succinate, Amlodipine Besylate and chlorthalidone have been reported. However, no chromatographic techniques have been earlier presented for the Metoprolol Succinate, Amlodipine Besylate and chlorthalidone combination addressing in the occurrence of their degradation products while assessing greenness.

The current study objective is to create an environmentally responsible method of examining this particular drug. With an emphasis on pollution avoidance, sustainable industrial ecology, controlling pollution, and environmental safety, this focus tackles environmental, social, and economic goals.

This work aim is to develop stability signaling HPLC method that has only just been developed in terms of how it effects the environment by examining its greenness characteristics employing the AGREE standards. This investigation, particularly took into account elements including solvent consumption, chemical substances, the use of energy, and trash formation, demonstrated the procedures' conservation of the environment.

IDENTIFICATION OF DRUG

1) Determination of Melting Point

Table. 01

Name of drug	Standard melting point	Observed melting point
Metoprolol Succinate	136-140°C	137-141°C
Amlodipine Besylate	195-204°C	196-205°C
Chlorthalidone	216°C to 222°C	217°C to 223°C

2) Solubility of the Drug

Table. 02

SOLVENT	SOLUBILITY		
	Metoprolol Succinate	Amlodipine Besylate	Chlorthalidone
Water	Freely Soluble	Slightly soluble	Slightly soluble
2-Propanol	Slightly soluble	Slightly soluble	Insoluble
Ethanol	Sparingly Soluble	Sparingly Soluble	Slightly soluble
Methanol	Freely Soluble	Freely Soluble	insoluble

DETERMINATION OF WAVELENGTH FOR MAXIMUM ABSORBANCE:

The sensitivity of HPLC method that uses UV detection depends on proper selection of detection wavelength. An ideal wavelength is that gives good response for drugs those are to be detected.

When the individual solution, having concentration of 10 µg/ml of Metoprolol Succinate and 50 µg/ml of Amlodipine Besylate and 50 µg/ml of Chlorthalidone were scanned between 200-800 nm, it was observed that at 242 nm, adequate signal of all the components can be generated and hence it was selected as analytical wavelength for further method development.

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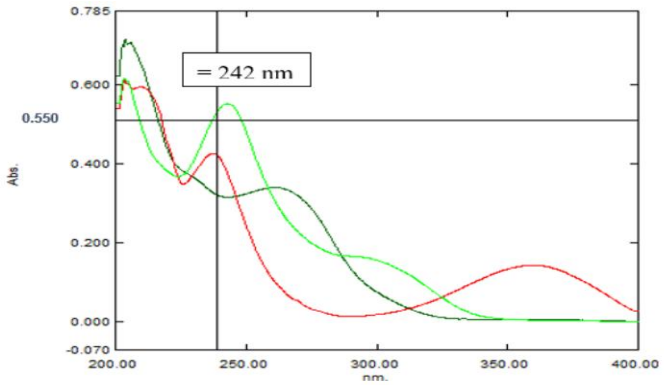

Fig. 04 Wavelength

SELECTION OF MOBILE PHASE:

Table. 03

Trial No.	**Mobile Phase**	**Flow Rate (ml/min)**	**Ratio (V/V)**	**Retention Time (min)**	**Remark**
01	Water : Acetonitrile	1.0	50 : 50	10	Only One Peak Observed
02	Water : Methanol	1.0	30 : 70	15	Only Two Peak Observed
03	0.02M Potassium Dihydrogen Phosphate : Methanol	1.0	45 : 55	15	Separate Achieved With Interference
04	0.02M Potassium Dihydrogen Phosphate : Acetonitrile	1.0	45 : 55	15	Separate Achieved With Interference

OPTIMIZED CHROMATOGRAPHIC CONDITION

Table. 04

Stationary phase	Hibar ODS C18 (250*4.6 mm, 5µm)
Mobile Phase	0.02M Potassium Dihydrogen Phosphate : Acetonitrile (45:55 v/v)
Detection wavelength	242 nm
Flow rate	1.0 ml/min
Run time	15 minutes
Retention Time	Metoprolol Succinate 7.811 min, Amlodipine Besylate 10.651 min, & Chlorthalidone 13.574 min

❖ PREPARATION OF STOCK SOLUTION

PREPARATION OF STANDARD STOCK SOLUTION OF METOPROLOL SUCCINATE

Master Stock Solution: - Accurately Weight 500mg Metoprolol Succinate Dissolved in 100ml Methyl alcohol (5000 µg/ml). Further dilute 1ml of this Solution 10ml with Methyl alcohol (500µg/ml).

PREPARATION OF STANDARD STOCK SOLUTION OF AMLODIPINE BESYLATE

Master Stock Solution: - Accurately Weight 50mg Amlodipine besylate Dissolved in 100ml Methyl alcohol (500µg/ml). Further dilute 1ml of this Solution 10ml with Methyl alcohol (50µg/ml). Dilute 1ml of this Solution 5ml with Methyl Alcohol (5µg/ml).

PREPARATION OF STANDARD STOCK SOLUTION OF CHLORTHALIDONE

Master Stock Solution:- Accurately Weight 125mg Chlorthalidone Dissolved in 100ml Methyl alcohol (1250 µg/ml). Further dilute 1ml of this Solution 10ml with Methyl alcohol (125µg/ml). Dilute 1ml of this Solution 12.5ml with Methyl Alcohol (12.5µg/ml)

PREPARATION OF STANDARD STOCK SOLUTION OF MIXTURE

Master Stock Solution: - Weight accurately about 500mg of Metoprolol Succinate, 50mg of Amlodipine Besylate and 125mg of Chlorthalidone and transfer into a 100ml volumetric flask. Mark up the volume of the flask to the mark with Methanol. (5000µg/ml Metoprolol Succinate+50µg/ml Amlodipine Besylate+1250µg/ml of Chlorthalidone). Take 1ml of this solution to 10ml volumetric flask and mark up to the volume with Methanol,

Resulting Solution Prepare 500µg/ml Metoprolol Succinate+50µg/ml Amlodipine Besylate+125µg/ml Chlorthalidone. Take 1ml from master stock solution and make up to 10 ml with methyl alcohol to Resulting Solution Prepare 50µg/ml Metoprolol Succinate+5µg/ml Amlodipine Besylate+12.5 µg/ml Chlorthalidone.

FORCED DEGRADATION STUDIES

ACID DEGRADATION

Weigh accurately about 500mg of Metoprolol Succinate, 50mg of Amlodipine Besylate and 125mg of Chlorthalidone and transfer in to a 100ml volumetric flask. Make up the volume of the flask to the mark with Methanol. (5000µg/ml Metoprolol Succinate+500µg/ml Amlodipine Besylate+1250µg/ml of Chlorthalidone). Transfer 1ml of above Solution in to 10ml Volumetric flask, add 5mL 0.5N HCl **(0-hour sample as STD).** Solution was placed under reflux condition at 60°C for 1 hour. After that, cool the solution and neutralize with 1N NaOH. Transfer 1ml of above Solution into 10ml volumetric flask, make up the volume with methanol.

ACID DEGRADATION TRIAL

Table. 05

Trials	Condition	% Degradation		
		Metoprolol Succinate	Amlodipine Besylate	Chlorthalidone
Trials 1	0.05N HCl, refluxed at 60°C for 30 minutes	4.02	3.05	5.05
Trials 2	0.1N HCl, refluxed at 60°C for 45 minutes	8.13	5.28	7.96
Trials 3	0.5N HCl, refluxed at 60°C for 60 minutes	17.96	16.26	19.05

Acid Controlled

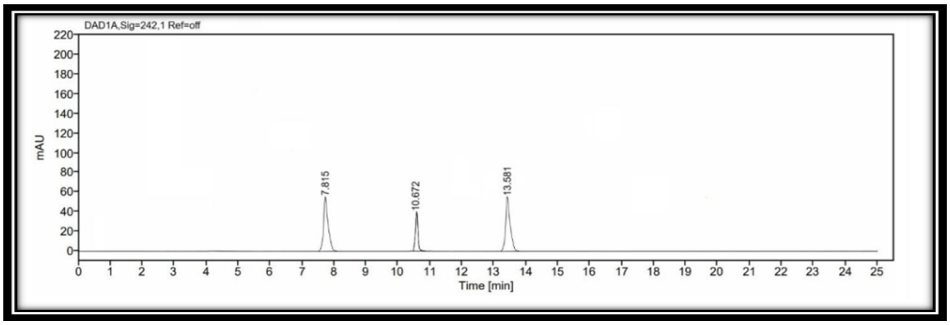

Fig. 05 Acid Controlled

Chromatogram of Metoprolol Succinate+ Amlodipine Besylate + Chlorthalidone
(50+5+12.5 µg/ml) Acid Controlled sample

BASE DEGRADATION

Weigh accurately about 500mg of Metoprolol Succinate, 50mg of Amlodipine Besylate and 125mg of Chlorthalidone and transfer in to a 100ml volumetric flask. Make up the volume of the flask to the mark with Methanol. (5000µg/ml Metoprolol Succinate+500µg/ml Amlodipine Besylate+1250µg/ml of Chlorthalidone). Transfer 1ml of above Solution in to 10ml Volumetric flask, add 5mL 0.5N NaOH **(0-hour sample as STD).** Solution was placed under reflux condition at 60°C for 1 hour. After that, cool the solution and neutralize with 1N HCl. Transfer 1ml of above Solution into 10ml volumetric flask, make up the volume with methanol.

BASE DEGRADATION TRIAL

Table. 06

Trials	Condition	% Degradation		
		Metoprolol Succinate	Amlodipine Besylate	Chlorthalidone
Trials 1	0.05N NaOH, refluxed at 60°C for 30 minutes	5.89	8.99	6.64
Trials 2	0.1N NaOH, refluxed at 60°C for 45 minutes	7.77	12.52	10.26
Trials 3	0.5N NaOH, refluxed at 60°C for 60 minutes	15.96	24.41	17.89

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Base Controlled

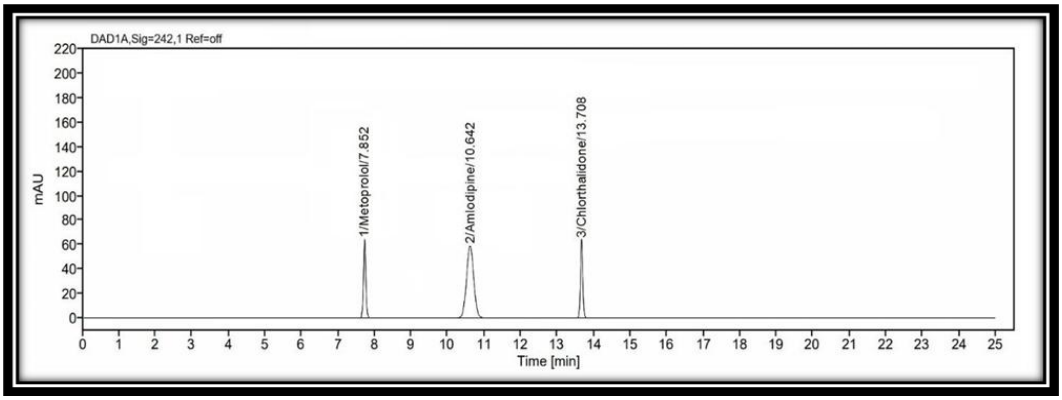

Fig. 06

Chromatogram of Metoprolol Succinate + Amlodipine Besylate + Chlorthalidone (50+5+12.5 µg/ml) Base Controlled of sample

OXIDATIVE DEGRADATION

Weigh accurately about 500mg of Metoprolol Succinate, 50mg of Amlodipine Besylate and 125mg of Chlorthalidone and transfer in to a 100ml volumetric flask. Make up the volume of the flask to the mark with Methanol. (5000µg/ml Metoprolol Succinate+500µg/ml Amlodipine Besylate+1250µg/ml of Chlorthalidone). Transfer 1ml of above Solution in to 10ml Volumetric flask, add 5mL 6% H_2O_2 **(0-hour sample as STD).** Solution was placed under reflux condition at 60°C for 1 hour. After that, cool the solution and with methanol. Transfer 1ml of above Solution into 10ml volumetric flask, make up the volume with methanol.

OXIDATIVE DEGRADATION TRIAL

Table. 07

Trials	Condition	% Degradation		
		Metoprolol Succinate	Amlodipine Besylate	Chlorthalidone
Trials 1	3% H_2O_2, refluxed at 60°C for 60 minutes	7.95	10.28	8.88
Trials 2	6% H_2O_2, refluxed at 60°C for 60 minutes	14.26	16.99	13.24

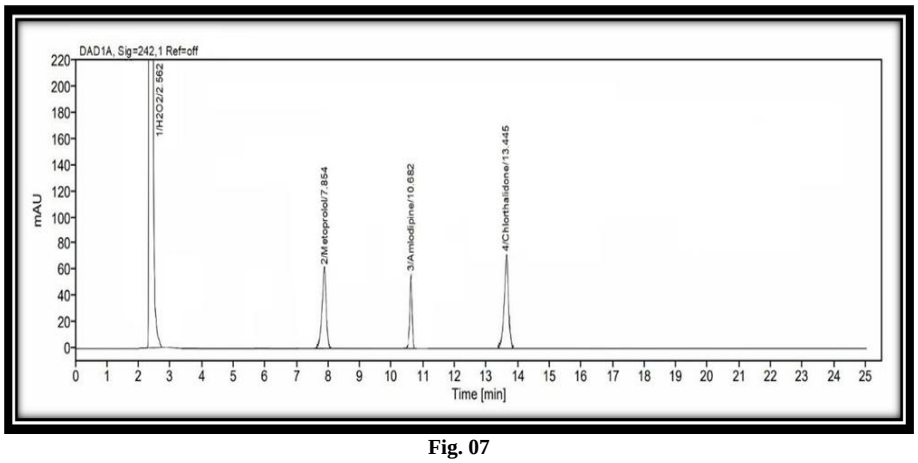

Fig. 07

Chromatogram of Metoprolol Succinate+Amlodipine Besylate+Chlorthalidone (50+5+12.5µg/ml) Oxidative Controlled sample

THERMAL DEGRADATION

Weigh accurately about 500mg of Metoprolol Succinate, 50mg of Amlodipine Besylate and 125mg of Chlorthalidone and transfer in to Petri dish and was kept in preheated oven at 80°C for 2 Hours. After that transfer in to a 100 ml volumetric flask. Make up the volume of the flask to the mark with Methanol. (5000µg/ml Metoprolol Succinate+500µg/ml Amlodipine Besylate+1250µg/ml of Chlorthalidone). Transfer 1ml of above Solution in to 10

ml volumetric flask; make up the volume with methanol.

THERMAL DEGRADATION TRIAL

Table. 08

Trials	Condition	% Degradation		
		Metoprolol Succinate	Amlodipine Besylate	Chlorthalidone
Trials 1	40ºC for 2 hr	5.42	9.85	9.49
Trials 2	80ºC for 2 hr	13.26	15.28	16.28

Analytical Method Validation:

LINEARITY & RANGE

- **Preparation of solution for linearity studies.**

1. Weight accurately about 500mg Metoprolol Succinate, 50mg of Amlodipine Besylate, & 125mg of Chlorthalidone and transfer into a 100 ml volumetric flask.
2. Make up the volume of the flask to the mark with methanol. (5000µg/ml metoprolol succinate + 500µg/ml Amlodipine Besylate + 1250 µg/ml of Chlorthalidone)
3. Transfer 1 ml of this solution to 10ml volumetric flask, and make up the volume with methanol, the resulting solution gives (500µg/ml metoprolol succinate + 50µg/ml amlodipine besylate + 125 µg/ml of Chlorthalidone)
4. Various aliquots from this stock solution were transferred to another 10ml volumetric flask and volume was raised to the mark with mobile phase to give final solutions.

Table. 09

Concentration of stock solution	Volume taken (µg/ml)	Dilution volume with methanol	Final Concentration of Metoprolol Succinate+Amlodipine besylate+ Chlorthalidone
500µg/ml Metoprolol succinate + 50µg/ml Amlodipine besylate + 125 µg/ml of Chlorthalidone	0.5 ml	Up to 10 ml	25 + 2.5 + 6.25 µg/ml
	1.0 ml	Up to 10 ml	50 + 5 + 12.5 µg/ml
	1.5 ml	Up to 10 ml	75 + 7.5 + 18.75 µg/ml
	2.0 ml	Up to 10 ml	100 + 10 + 25 µg/ml
	2.5 ml	Up to 10 ml	125 + 12.5 + 31.25 µg/ml

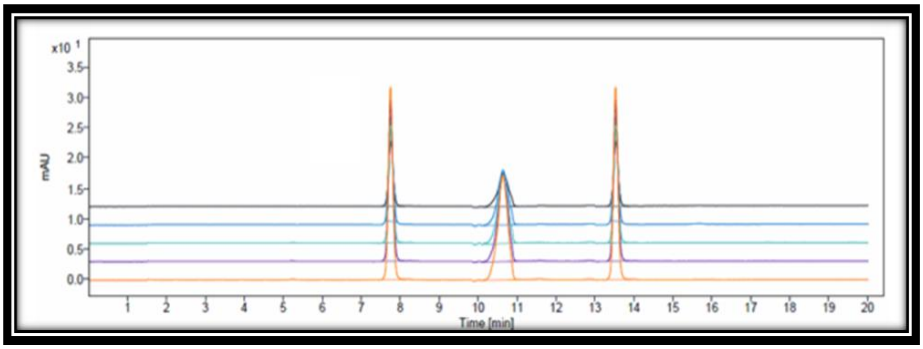

Fig. 08

Table. 10

Concentration (µg/ml)	Metoprolol Succinate
0	0
25	79863
50	161245
75	257464
100	340574
125	435896

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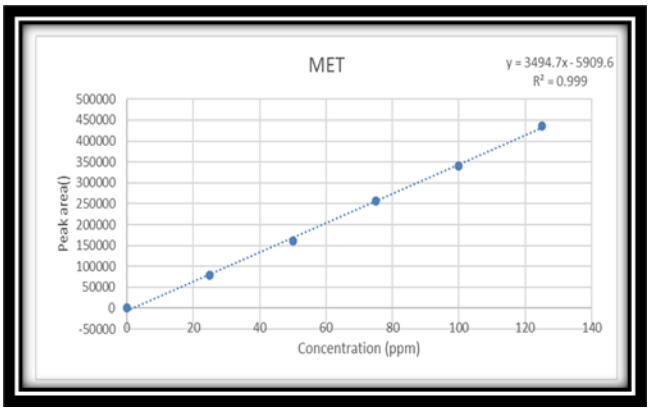

Fig. 09

Table. 11

Concentration (µg/ml)	Amlodipine Besylate
0	0
2.5	8475
5	15124
7.5	23745
10	32246
12.5	40578

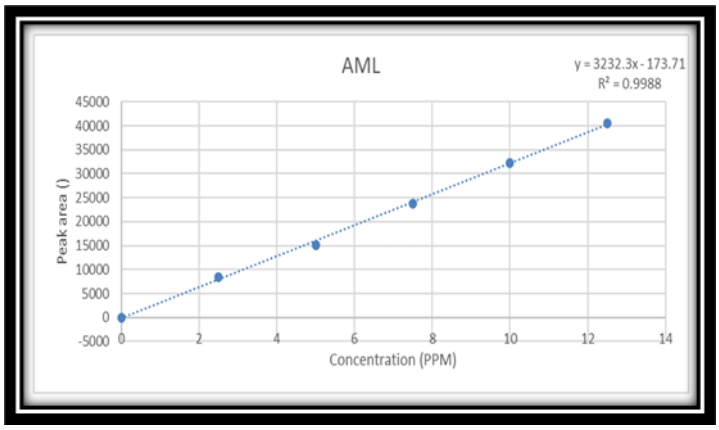

Fig. 10

Table. 12

Concentration (µg/ml)	Chlorthalidone
0	0
6.25	21475
12.5	42814
18.75	62574
25	86754
31.25	108486

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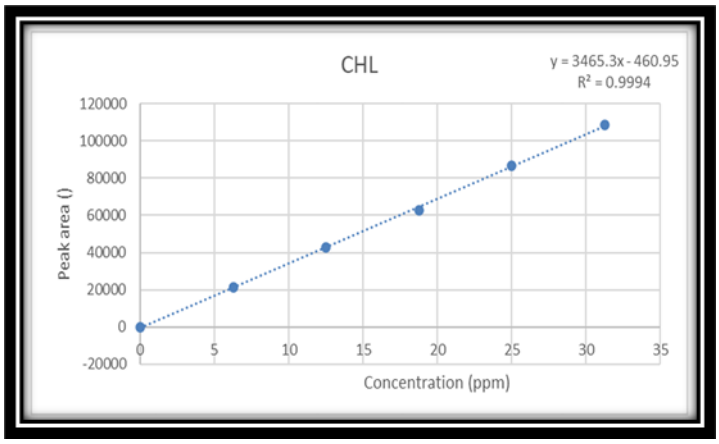

Fig. 11

REPEATABILITY:

➢ **Preparation of solution.**

✓ Prepare standard working solution of mixture having concentration of Metoprolol Succinate 25, 50, 75, 100 & 125µg/ml, Amlodipine Besylate 2.5, 5, 7.5, 10 & 12.5µg/ml, Chlorthalidone 6.25, 12.5, 18.75, 25 & 31.25µg/ml were injected at volume of 20µl into column by employing optimized chromatographic condition.

✓ Each standard mixture was injected 5 times and peak area was monitored. Each concentration was monitored for repeatability by RSD.

Table. 13

Metoprolol Succinate					
Concentration (µg/ml)	25	50	75	100	125
Area 1	79800	161245	257465	340600	435786
Area 2	80450	162100	258900	342150	438200
Area 3	79200	159850	255800	338900	433500
Area 4	80120	161700	259100	343200	437100
Area 5	79550	160500	256500	340100	434900
Mean	79824	161079	257553	340990	435897.2
SD	485.93	908.48	1448.89	1698.68	1837.32
RSD	0.61	0.56	0.56	0.50	0.42
Amlodipine Besylate					
Concentration (µg/ml)	2.5	5	7.5	10	12.5
Area 1	8475	15124	23745	32246	40578
Area 2	8390	14980	23550	32500	40200
Area 3	8550	15250	23900	31950	40900
Area 4	8420	15050	23680	32100	40400
Area 5	8510	15180	23820	32380	40577
Mean	8469	15116.8	23739	32235.2	40531
SD	65.04	106.02	133.90	218.42	258.46
RSD	0.77	0.70	0.56	0.68	0.64
Chlorthalidone					
Concentration (µg/ml)	6.25	12.5	18.75	25	31.25
Area 1	21475	42814	62574	86754	108486
Area 2	21680	43150	62900	87200	109203
Area 3	21300	42500	62100	86100	107806
Area 4	21550	42900	62750	86951	108752
Area 5	21420	42700	62400	86571	108126
Mean	21485	42812.8	62544.8	86715.2	108474.6
SD	142.13	240.77	311.42	415.75	542.26
RSD	0.66	0.56	0.50	0.48	0.50

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LIMIT OF DETECTION (LOD) & LIMIT OF QUANTIFICATION (LOQ)

Table. 14

Metoprolol Succinate	**Slope**	**Intercept**
	3465.2	5909.6
	3520.8	5911.8
	3488.5	5905.7
	3505.4	5913.6
	3493.6	5915.8
Mean (S)	3494.7	
SD (σ)		3.9
	LOD	9.5
	LOQ	23.9
Amlodipine Besylate	**Slope**	**Intercept**
	3215.5	172.61
	3250	170.8
	3232.3	168.6
	3244.2	175.9
	3219.5	169.6
Mean (S)	3232.3	
SD (σ)		2.9
	LOD	1
	LOQ	2.2
Chlorthalidone	**Slope**	**Intercept**
	3435	461.2
	3470.5	458.5
	3456.3	463.48
	3482.1	460.8
	3437.6	465.9
Mean (S)	3465.3	
SD (σ)		2.8
	LOD	1.9
	LOQ	5.6

ACCURACY:

Accuracy of the analytical method has been performed by spiking of Placebo with the standard. Spiking of the sample was performed at 50 %, 100 % and 150 % of the target concentration. Composition of placebo: Directly Compressible Lactose (100 mg), Talc (2 mg) and Magnesium stearate (2 mg).

Table. 15

Amount of Placebo			**104 mg**			**104 mg**			**104 mg**			**104 mg**		
Concentration of stock solution (Standard)			500µg/ml Metoprolol Succinate + 50µg/ml Amlodipine Besylate + 125µg/ml of Chlorthalidone.											
Volume of Standard taken in ml from stock solution of standard			----			0.5 ml			1.0 ml			1.5 ml		
Diluted (Up to 10 ml)			Mobile phase			Mobile phase			Mobile phase			Mobile phase		
Concentration corresponding to standard solution taken			----			25+2.5+6.25 µg/ml			50+5+12.5 µg/ml			75+7.5+18.75 µg/ml		
Identification			Unspiked			50 % Spiked			100 % Spiked			150 % Spiked		
Metoprolol Succinate														
50%			100%						150%					
Amount of drug added	Amount of drug recovered	% Recovery	Amount of drug added	Amount of drug recovered	% Recovery				Amount of drug added	Amount of drug recovered	% Recovery			
25	24.72	98.88	50	49.65	99.3				75	74.45	99.27			
25	25.08	100.32	50	50.15	100.3				75	75.12	100.16			
25	24.85	99.4	50	49.88	99.76				75	74.75	99.67			
Mean	24.88	99.53		49.88	99.79					74.77	99.70			
SD	0.18	0.73		0.25	0.50					0.34	0.45			
Amlodipine Besylate														
50%			100%						150%					
Amount of drug	Amount of drug	% Recovery	Amount of drug added	Amount of drug	% Recovery				Amount of drug added	Amount of drug	% Recovery			

added	recovered			recovered			recovered	
2.5	2.47	98.8	5	4.96	99.2	7.5	7.42	98.93
2.5	2.51	100.4	5	5.04	100.8	7.5	7.55	100.67
2.5	2.49	99.6	5	4.99	99.8	7.5	7.48	99.73
Mean	2.49	99.6		5	99.93		7.48	99.78
SD	0.02	0.80		0.04	0.81		0.07	0.87
Chlorthalidone								
50%			100%			150%		
Amount of drug added	Amount of drug recovered	% Recovery	Amount of drug added	Amount of drug recovered	% Recovery	Amount of drug added	Amount of drug recovered	% Recovery
6.25	6.18	98.88	12.5	12.38	99.04	18.75	18.52	98.77
6.25	6.27	100.32	12.5	12.55	100.4	18.75	18.78	100.16
6.25	6.22	99.52	12.5	12.48	99.84	18.75	18.68	99.63
Mean	6.22	99.57		12.47	99.76		18.66	99.52
SD	0.05	0.72		0.09	0.68		0.13	0.70

INTRA-DAY & INTER-DAY PRECISION

- Method precision was determined by performing intraday and interday precision.
- Mixture that represents overall rang of Metoprolol Succinate (25, 75 & 125µg/ml), Amlodipine Besylate (2.5, 7.5 & 12.5µg/ml), Chlorthalidone (6.5, 18.75 & 31.25µg/ml) were analysed on same day at different time interval for intraday precision.
- Mixture that represents overall rang of Metoprolol Succinate (25, 75 & 125µg/ml), Amlodipine Besylate (2.5, 7.5 & 12.5µg/ml), Chlorthalidone (6.5, 18.75 & 31.25µg/ml) were analysed on different day at different time interval for interday precision.

Table. 16

Intraday Precision			
Metoprolol Succinate			
Concentration (µg/ml)	25	75	125
Area 1	79942	257652	435906
Area 2	80150	258400	437025
Area 3	79720	256482	435876
Mean	79937.33	257511.33	436269
SD	215.04	966.71	654.89
RSD	0.27	0.38	0.15
Intraday Precision			
Amlodipine Besylate			
Concentration (µg/ml)	2.5	7.5	12.5
Area 1	6900	20800	36900
Area 2	6985	21050	37250
Area 3	6860	20620	36650
Mean	6815	20823.33	36933.33
SD	63.84	215.95	301.36
RSD	0.92	1.04	0.82
Intraday Precision			
Chlorthalidone			
Concentration (µg/ml)	6.25	18.75	31.25
Area 1	18900	61825	108284
Area 2	19150	62540	109604
Area 3	18720	61350	107380
Mean	18923.33	61905	108422.67
SD	215.95	599.02	1118.47
RSD	1.14	0.97	1.03

Table. 17

Interday Precision			
Metoprolol Succinate			
Concentration (µg/ml)	25	75	125
Area 1	69785	264359	440080
Area 2	70550	266100	443200

Area 3	69200	262800	437524
Mean	69845	264419.67	440268
SD	677	1650.84	2842.67
RSD	0.97	0.62	0.65
Interday Precision			
Amlodipine Besylate			
Concentration (µg/ml)	2.5	7.5	12.5
Area 1	7500	22600	38541
Area 2	7580	22850	38900
Area 3	7440	22420	38150
Mean	7506.67	22623.33	38530.33
SD	70.24	215.95	375.11
RSD	0.94	0.95	0.97
Interday Precision			
Chlorthalidone			
Concentration (µg/ml)	6.25	18.75	31.25
Area 1	20531	56481	109700
Area 2	20800	57200	110900
Area 3	20350	55950	108600
Mean	20560.33	56543.67	109733.33
SD	226.43	627.35	1150.36
RSD	1.10	1.11	1.05

ROBUSTNESS:

Following parameters were altered one by one for determination of robustness of the method and their effect was observed by comparing with the standard preparation.

A. Mobile phase flow rate (±0.1 ml/min), optimized flow rate 1 ml/min.
B. Mobile phase composition (±2ml), in optimized ratio.

Three determinations of Metoprolol Succinate, Amlodipine Besylate, & Chlorthalidone is 50+5+12.5µg/ml for each alteration was carried out and RSD was measured.

Table. 18

Parameter	**Level of change**	**Metoprolol Succinate 50µg/ml**	**Amlodipine Besylate 5µg/ml**	**Chlorthalidone 12.5µg/ml**
Flow rate of Mobile phase	0.9 ml/min	141536	13942	47200
	1 ml/min	140850	14090	46850
	1.1 ml/min	139980	13820	46500
	Mean	140788.67	13950.67	46850
	SD	779.81	135.21	350
	RSD	0.55	0.97	0.75
Composition of Mobile phase	43:57	141608	13850	46900
	45:55	142300	14010	47250
	47:53	140900	13720	46550
	Mean	141602.67	13860	46900
	SD	700.02	145.26	350
	RSD	0.49	1.05	0.75

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