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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF METOPROLOL TARTRATE, AMLODIPINE BESYLATE AND CHLORTHALIDONE IN SYNTHETIC MIXTURE

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ABSRACT

Simple, specific, accurate, precise and reproducible and robust method have been developed and validated for the Simultaneous Estimation of Metoprolol tartrate, Amlodipine besylate and Chlorthalidone in synthetic mixture. The Reverse Phase High Performance Liquid Chromatography, the chromatographic system was equipped with Cosmosil C_{18} (250 mm × 4.6 mm, 5 µm) and UV detector set at 222 nm, in conjunction with a mobile phase of Phosphate Buffer pH 2.5: ACN: Methanol in the ration of (50:30:20%v/v), at a flow rate of 1.5 mL/min. Retention time was noted to be 1.44, 1.33 and 1.13 for Metoprolol Tartrate, Amlodipine Besylate and Chlorthalidone respectively. The various analytical parameters, including specificity, linearity, LOD, LOQ, precision, accuracy, and robustness, were determined ICH guidelines. The method was linear over the range of 245.53-736.60 µg/ml, 25.06-75.18 µg/ml and 24.81-74.44 µg/ml for Metoprolol Tartrate, Amlodipine Besylate and Chlorthalidone respectively. The % recoveries for Metoprolol Tartrate was noted to be 101.7% - 100.5%, 98.4% - 101.3% for Amlodipine Besylate and 100.7% - 99.8% for Chlorthalidone. The developed RP-HPLC method was found to be specific, accurate, precise, robust for simultaneous estimation of Metoprolol Tartrate, Amlodipine Besylate and Chlorthalidone in synthetic mixture and stable for routine

Keywords: Metoprolol tartrate, Amlodipine besylate, Chlorthalidone, RP-HPLC method, Method Development

INTRODUCTION

A sustained rise in systemic arterial pressure above a certain threshold is referred to as hypertension. a cardiovascular condition that advances over other syndromes with intricately linked etiologies [1]. The millimeter of mercury (mm Hg) is the unit of measurement for blood pressure. A blood pressure reading of 120 over 80 mm Hg is conceivable. The borderline for good blood pressure is 120/80 mm Hg [2]. For the blood to flow throughout its own tissues, the body needs this pressure. Healthy arteries can enlarge to accommodate more blood because they are elastic. This suggests that, despite being higher than usual, the blood pressure is not high enough to be categorized as hypertension [3]. If the diastolic blood pressure is 90 or higher, or the systolic blood pressure is 140 or higher, it is deemed high blood pressure. Blood pressure levels associated with a higher risk of cardiovascular disease are known as hypertension. Treatment is generally acknowledged to be at least taken into consideration above a cut-off of 140/90 mm Hg [4]. This would equate to most wealthy countries having an adult prevalence of hypertension of roughly 20%. Peripheral vascular disease, heart failure, atrial fibrillation, stroke, and renal impairment are all linked to higher risk of hypertension [5].

Metoprolol is a β-blocker that is used to treat angina, hypertension and to lower myocardial infraction mortality [6]. Amlodipine is a calcium channel blocker used to treat hypertension [7]. Chlorthalidone is a diuretic used to treat hypertension or edema caused by heart failure, renal failure, hepatic cirrhosis, estrogen therapy and other conditions [8].

METHOD AND MATERIALS:

Shimadzu HPLC and LC 2010 CHT model LC Solution software was used. Shimadzu UV-1800 double-beam UV-VIS spectrophotometer Instrument was used. Cosmosil C_{18} (250 mm × 4.6 mm internal diameter × 5 micron particle size). API was obtained as gift sample from TORRENT PHARMACEUTICAL LTD. Acetonitrile, methanol, water, phosphate buffer of HPLC grade from Merck Life Science Pvt. Ltd./Advent Chembio Pvt. Ltd./Thermo Fisher Scientific Pvt. Ltd./Spectrochem Pvt. Ltd.

IDENTIFICATION AND WAVELENGTH SELECTION: Agilent Cary 630 FTIR Spectrometer and Software: MicroLab ExpertFT-IR Instrument was used. Spectra of Metoprolol tartrate, Amlodipine besylate and Chlorthalidone standard was obtained by FT-IR spectrophotometer. FTIR spectra were interpreted and results were co-related with

M.P., UV spectra and solubility to confirm identity of individual drugs. Wavelength was selected from the overlay spectra of above solutions.

SELECTION OF MOBILE PHASE: Various mobile phases were tried. Trial contains various mobile phases which consisted of Acetonitrile, methanol, water, phosphate buffer in different proportions with various pH and different volumes at flow rate 1 ml/min were tried. Chromatogram in optimized mobile phase is shown in Figure.

Preparation of standard solution:

Buffer Preparation: 6.8 g potassium dihydrogen orthophosphate in 1 L water pH adjusted to 2.5 with orthophosphoric acid.

Mobile phase preparation: Buffer: ACN: methanol 50:18:33 %v/v.

Diluent: Mobile phase.

Preparation of stock solution:

Metoprolol Stock solution (5000 ppm): About 95 mg of metoprolol succinate working standard (equivalent to 100 mg of metoprolol succinate) was accurately weighed and transferred into 20 ml volumetric flask. To this, 5 ml of diluent was added and dissolved by sonication. The solution was diluted up to the mark with diluent and used as a stock solution.

Amlodipine stock solution (500 ppm): About 14 mg of amlodipine besylate working standard (equivalent to 10 mg of amlodipine, molecular weight correction factor 0.721) was accurately weighed and transferred into 20 ml volumetric flask. To this, 10 ml of diluent was added and dissolved by sonication. The solution was diluted up to the mark with diluent and used as a stock solution.

Chlorthalidone stock solution (1250 ppm): About 25 mg of chlorthalidone working standard was accurately weighed and transferred into 20 ml volumetric flask. To this, 10 ml of diluent was added and dissolved by sonication. The solution was diluted up to the mark with diluent and used as a stock solution.

Mix standard solution (500 ppm Metoprolol + 50 ppm Amlodipine + 125 ppm Chlorthalidone): Pipette out 1 ml of each stock solution into 10 ml volumetric flask. Make volume up to the mark with diluent to get mixed standard solution.

Sample preparation

Synthetic mixture was prepared as per the per references. Then the synthetic mixture equivalent to one tablet was accurately weighed and taken into 100 ml volumetric flask containing 20 ml of diluent. The mixture was sonicated for 15 minute to dissolve the content. Then volume was made up to the mark with diluent with intermittent shaking. The resultant solution was filtered through

Whatman 0.45 μm membrane filter and used as a sample solution and the final concentration was 500 μg/ml, 125 μg/ml chlorthalidone and 50 μg/ml amlodipine.

Table 1: Optimized Chromatographic Conditions

Parameters	Chromatographic Condition
Mode of elution	Isocratic
Mobile Phase	Phosphate Buffer pH 2.5: ACN: Methanol (50:18:33)
Column	Cosmosil C-18 column (250 mm × 4.6 mm, 5.0μm)
Flow rate	1.5 ml/min
Runtime	20 min
Injection volume	20 μl
Detection wavelength	222 nm

Table 2: Structural interpretation of Amlodipine Besylate

Functional Group	Measured Frequency (cm⁻¹)	Reference Frequency (cm⁻¹)
O-H Stretch	3289.4	3300-3000
>C=O Stretch	1671.7	1680 – 1630
C-H Aliphatic Stretch	2927.8, 2928.0	3000-2840
N-H	1615.3	1650-1580
S=O Stretch	1090.2	1160-1120
C-Cl Stretch	613.1	785 – 540

Table 3: Structural interpretation of Metoprolol tartrate

Functional Group	Measured Frequency (cm⁻¹)	Reference Frequency (cm⁻¹)
O-H Stretch	3032.2	3300-3000
>C=O Stretch	1630	1680 – 1630
C-H Aliphatic Stretch	2871.9	3000-2840
N-H	1571.1	1650-1580
C-Cl Stretch	743.6	785 – 540

Table 4: Structural interpretation of Chlorthalidone

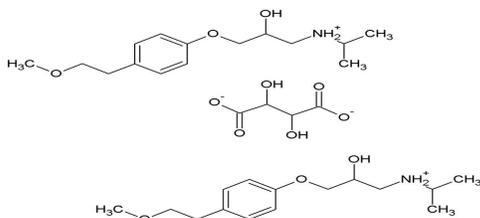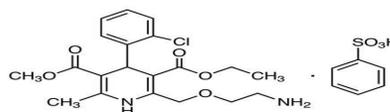| Functional Group	Measured Frequency (cm⁻¹)	Reference Frequency (cm⁻¹)
O-H Stretch	3083.9	3300-3000
N-H	1615.3	1650-1580
S=O Stretch	1123.8	1160-1120
C-Cl Stretch	784.5	785 – 540

Figure 1: Metoprolol Tartrate
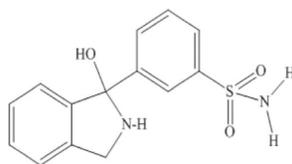
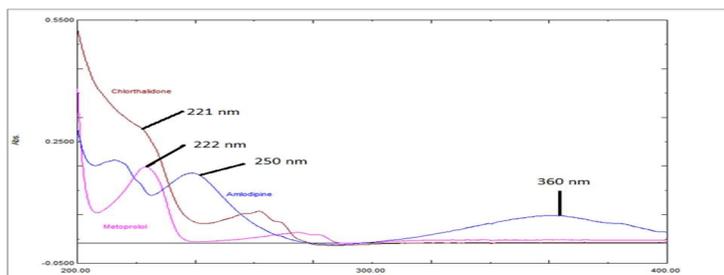**Figure 2: Amlodipine besylate**

Figure 3: Chlorthalidone

Figure 4: Overlay spectra of Amlodipine, Besylate Metoprolol tartrate, Chlorthalidone
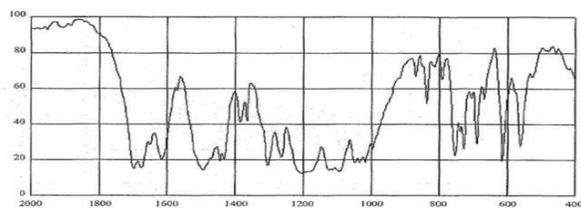
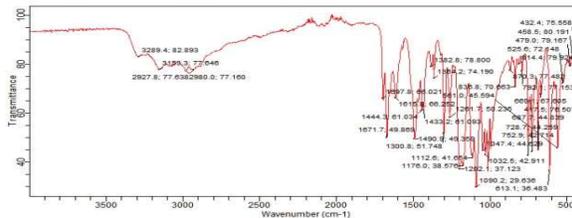

Figure 5: FTIR Spectra of Amlodipine Besylate Reference
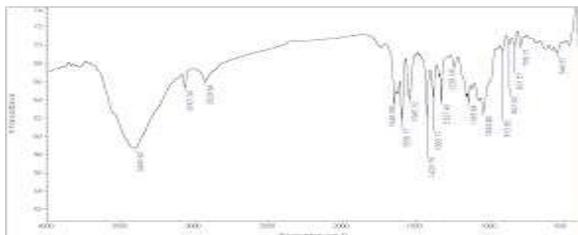

Figure 6: FTIR Spectra of API Sample

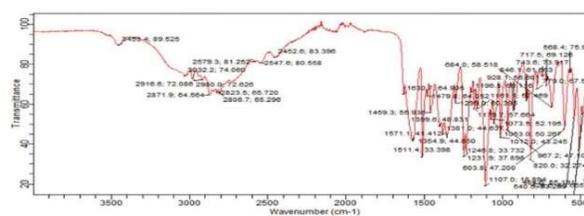**Figure 7: FTIR Spectra of Metoprolol tartrate Reference**
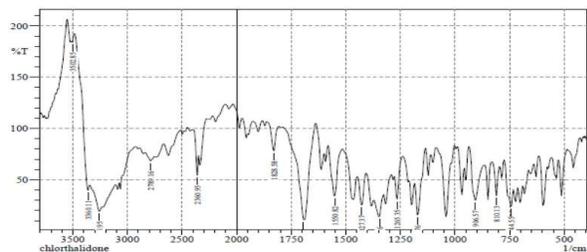

Figure 8: FTIR Spectra of API Sample

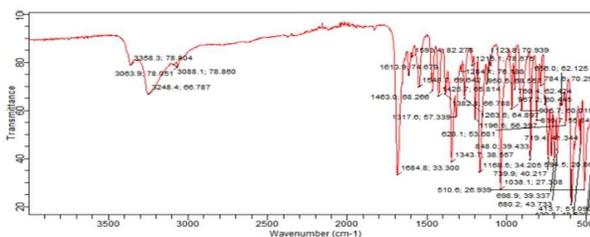**Figure 9: FTIR Spectra of Chlorthalidone References**

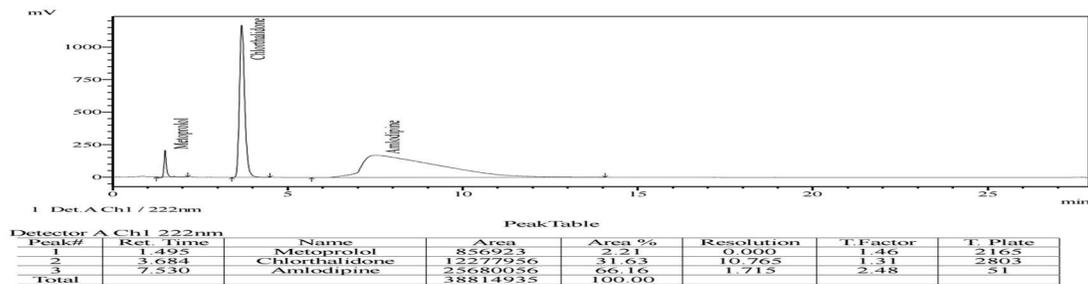**Figure 10: FT-IR Spectra of API Sample**

METHOD DEVELOPMENT

Trial 1

Peak#	Ret. Time	Name	Area	Area %	Resolution	T.Factor	T. Plate
1	1.495	Metoprolol	856923	2.21	0.000	1.46	2165
2	3.684	Chlorthalidone	12277956	31.63	10.765	1.31	2803
3	7.530	Amlodipine	25680056	66.16	1.715	2.48	51
Total			38814935	100.00			

Figure 11: Chromatogram of mixture in Water: Methanol (50:50%v/v)

Trial 2

Detector A Ch1 222nm

Peak#	Ret. Time	Name	Area	Area %	Resolution	T.Factor	T. Plate
1	1.282	Metoprolol	2388047	6.03	0.000	2.40	228
2	2.374	Chlorthalidone	11633696	29.40	3.833	1.12	1699
3	7.142	Amlodipine	25554341	64.57	2.643	3.72	72
Total			39576084	100.00			
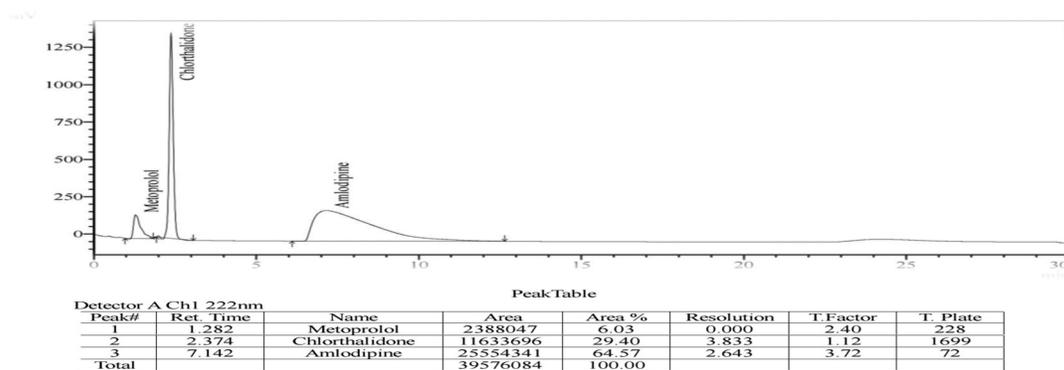
Figure 12: Chromatogram of mixture in Water: ACN (50:50%v/v)

Trial 3

Detector A Ch1 222nm

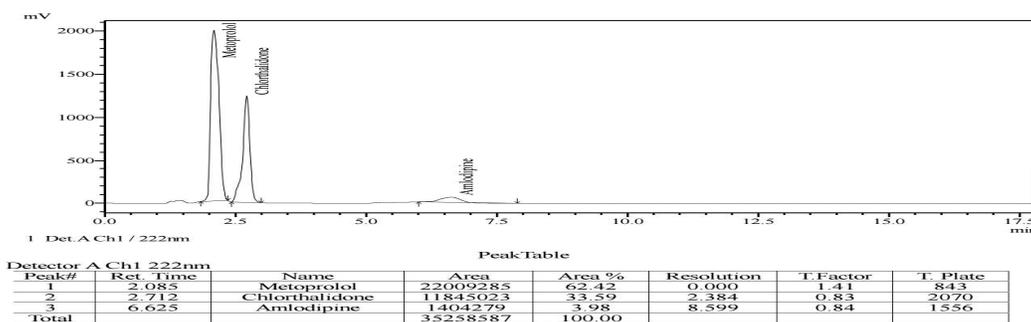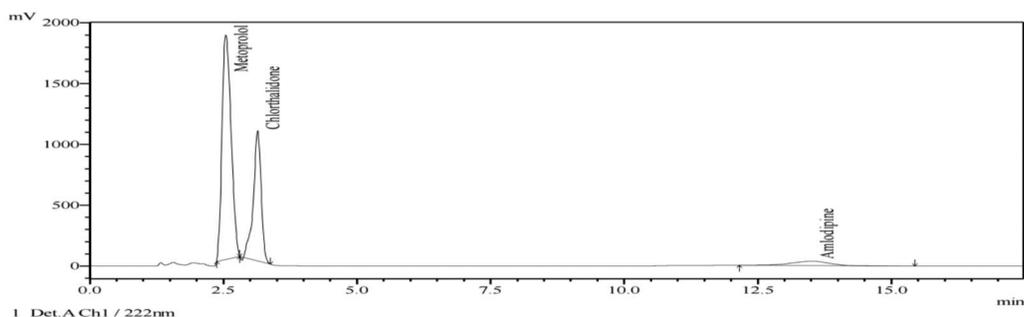| Peak#	Ret. Time	Name	Area	Area %	Resolution	T.Factor	T. Plate
1	1.899	Metoprolol	15171277	39.16	0.000	0.00	874
2	2.121	Chlorthalidone	20705282	53.44	0.815	0.00	873
3	3.383	Amlodipine	2869232	7.41	3.586	0.82	1054
Total			38745790	100.00			
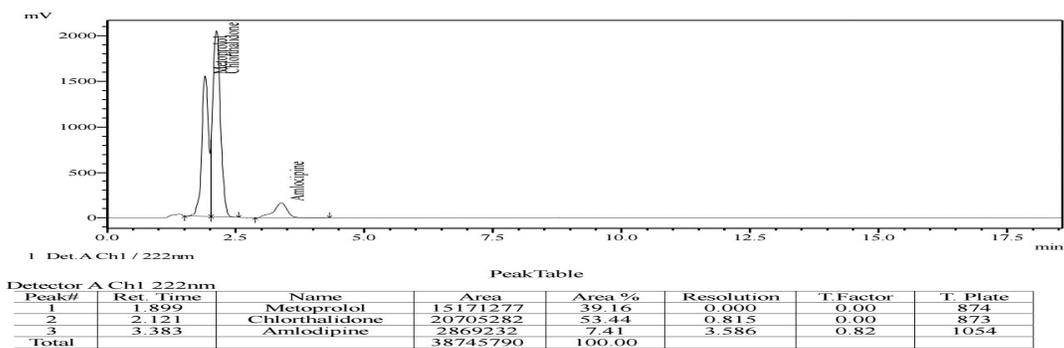
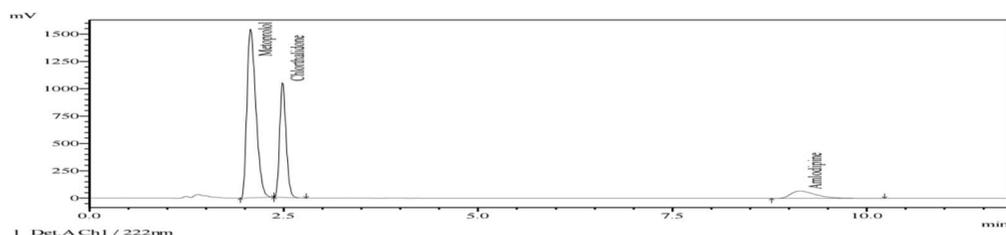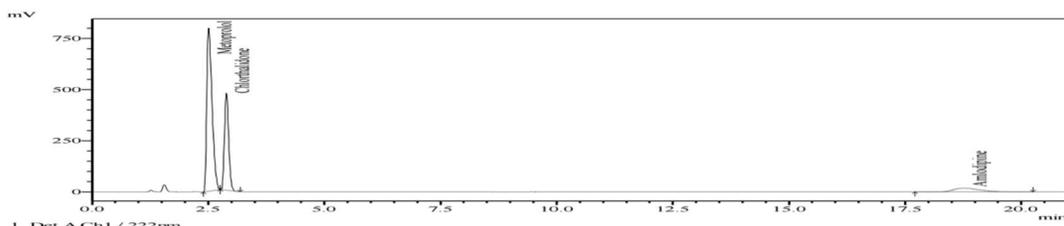**Figure 13: Chromatogram of mixture in Phosphate Buffer pH 3.5: ACN (50:50%v/v)**

Trial 4

Detector A Ch1 222nm

Peak#	Ret. Time	Name	Area	Area %	Resolution	T.Factor	T. Plate
1	2.085	Metoprolol	22009285	62.42	0.000	1.41	843
2	2.712	Chlorthalidone	11845023	33.59	2.384	0.83	2070
3	6.625	Amlodipine	1404279	3.98	8.599	0.84	1556
Total			35258587	100.00			

Figure 14: Chromatogram of mixture in Phosphate Buffer pH 3.5: ACN (60:40%v/v)

Trail 5

Detector A Ch1 222nm						
Peak#	Ret. Time	Name	Area	Area %	Resolution	T.Factor	T. Plate
1	2.540	Metoprolol	21857936	63.38	0.000	1.27	990
2	3.136	Chlorthalidone	10894712	31.59	2.041	0.84	2307
3	13.507	Amlodipine	1735371	5.03	13.146	0.84	1684
Total			34488018	100.00			

Figure 15: Chromatogram of mixture in Phosphate Buffer pH 3.5:ACN:Methanol (60:30:10%v/v)

Trail 6

Detector A Ch1 222nm						
Peak#	Ret. Time	Name	Area	Area %	Resolution	T.Factor	T. Plate
1	2.070	Metoprolol	12324310	62.21	0.000	1.44	1357
2	2.482	Chlorthalidone	6049006	30.53	2.115	1.24	3616
3	9.136	Amlodipine	1437859	7.26	18.046	1.60	4077
Total			19811175	100.00			

Figure 16: Chromatogram of mixture in Phosphate Buffer pH 2.5:ACN: Methanol (60:30:10%v/v)

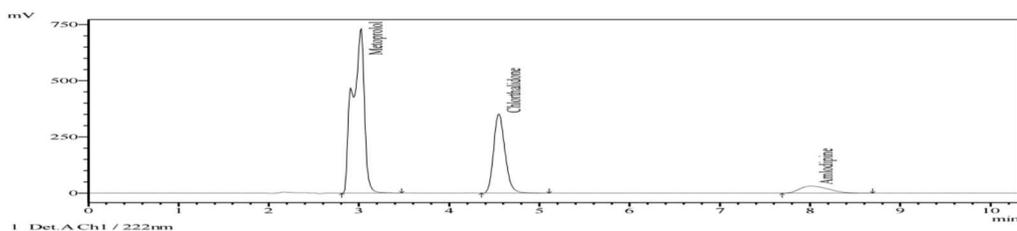**Trail 7**

Detector A Ch1 222nm						
Peak#	Ret. Time	Name	Area	Area %	Resolution	T.Factor	T. Plate
1	2.506	Metoprolol	6332310	63.01	0.000	1.68	2015
2	2.887	Chlorthalidone	3006178	29.91	1.891	1.22	4121
3	18.760	Amlodipine	711068	7.08	26.157	1.43	5270
Total			10049556	100.00			

Figure 17: Chromatogram of mixture in Phosphate Buffer pH 2.5:ACN:Methanol (60:20:20%v/v)

Trail 8

Detector A Ch1 222nm						
Peak#	Ret. Time	Name	Area	Area %	Resolution	T.Factor	T. Plate
1	3.016	Metoprolol	6656269	63.73	0.000	0.82	3016
2	4.545	Chlorthalidone	3092195	29.60	6.672	1.24	5799
3	8.006	Amlodipine	696419	6.67	8.361	1.40	2956
Total			10444883	100.00			

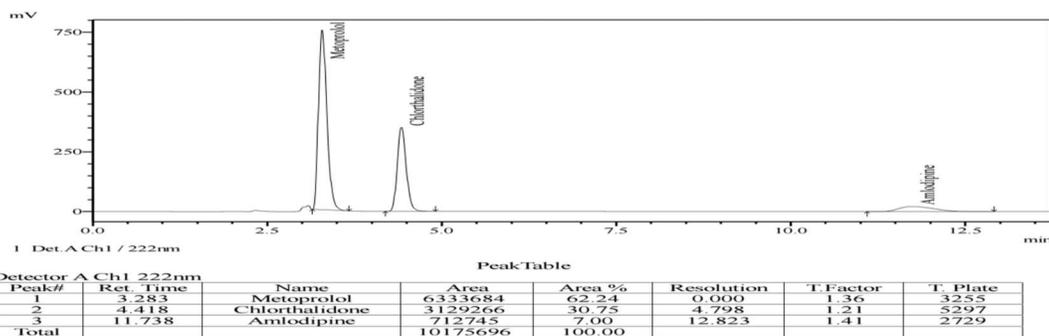**Figure 18: Chromatogram of mixture in Phosphate Buffer pH 2.5:ACN:Methanol (50:50%v/v)**

Trail 9

Figure 19: Chromatogram of mixture in Phosphate Buffer pH 2.5:ACN:Methanol (50:30:20%v/v)
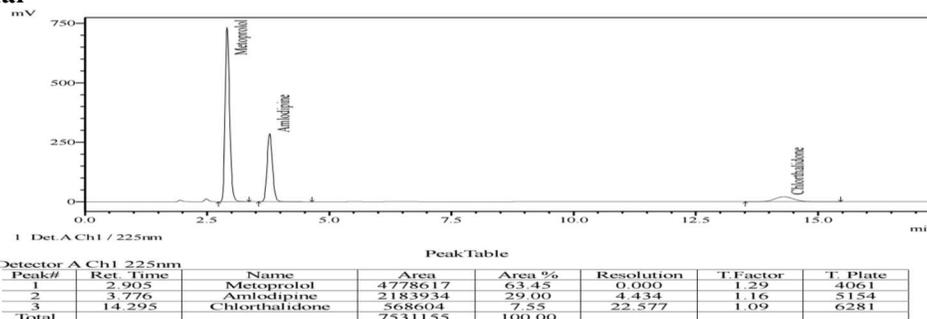
Trail 10 Final

Figure 20: Chromatogram of mixture in Phosphate Buffer pH 2.5:ACN:Methanol (50:30:20%v/v)
Comments: Peaks are well separated with resolution between metoprolol and chlorthalidone is more than 2. Tailing factor less than 2 and theoretical plate counts is more than 2000. Trial was finalized.

Table 5: Selection of Mobile Phase

Sr. no	Mobile phase composition	Inference
1	Water: Methanol (50:50%v/v)	Poor peak (Trial rejected)
2	Water: ACN (50:50%v/v)	Poor peak (Trial rejected)
3	Phosphate Buffer pH 3.5: ACN (50:50%v/v)	Two peak merged
4	Phosphate Buffer pH 3.5: ACN (60:40%v/v)	One poor peak (Trial rejected)
5	Phosphate Buffer pH 3.5: ACN : Methanol (60:30:10%v/v)	Improve resolution between two peaks
6	Phosphate Buffer pH 2.5: ACN : Methanol (60:30:10%v/v)	Poor resolution between two peaks (trail rejected)
7	Phosphate Buffer pH 2.5: ACN : Methanol (60:20:20%v/v)	One peak elutes very late (Trial rejected)
8	Phosphate Buffer pH 2.5: ACN: Methanol (50:50%v/v)	One peak split (Trial rejected)
9	Phosphate Buffer pH 2.5: ACN: Methanol (50:30:20%v/v)	Only one peak response (Trail rejected)
10	Phosphate Buffer pH 2.5: ACN: Methanol (50:30:20%v/v)	Peak Properly separated

Linearity and Range

Table 6: Linearity data for Metoprolol tartrate

Sr. No.	Concentration (ppm)	Area
1	245.53	2223546
2	368.30	3384595
3	491.07	4352586
4	613.83	5310391
5	736.60	6309482
Correlation (R)		0.99933
R^2		0.99866
Y-Intercept		277052.80
Slope		8225.08276

Table 7: Linearity data for Amlodipine besylate

Sr. No.	Concentration (ppm)	Area
1	25.06	259496
2	37.59	400218
3	50.12	526997
4	62.65	645433
5	75.18	764191
Correlation (R)		0.99932
R^2		0.99864
Y-Intercept		17425.00
Slope		10012.56166

Table 8: Linearity data for Chlorthalidone

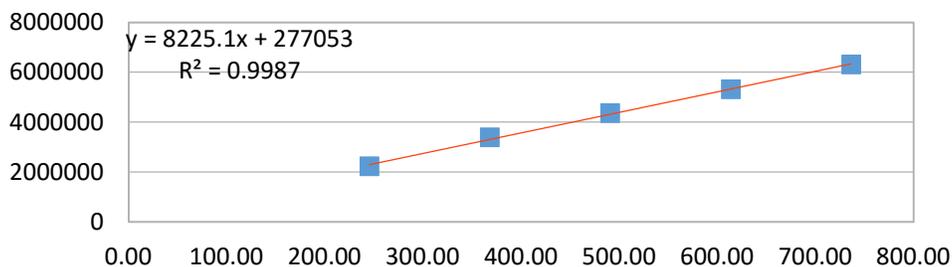| Sr. No.	Concentration (ppm)	Area
1	24.81	1030408
2	37.22	1558635
3	49.63	2054309
4	62.04	2518473
5	74.44	3070475
Correlation (R)		0.99967
R^2		0.99934
Y-Intercept		30471.20
Slope		40621.08890

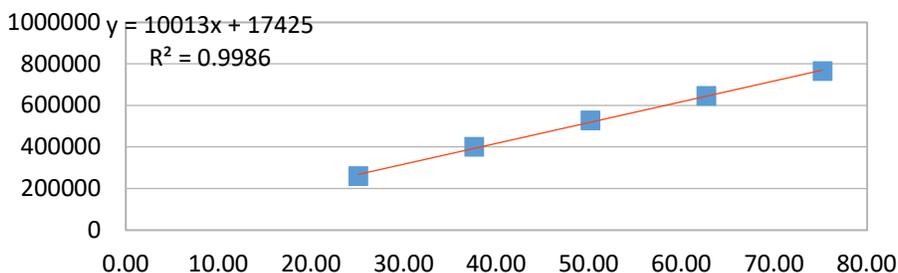**Figure 21: Calibration curve of Metoprolol Tartrate**

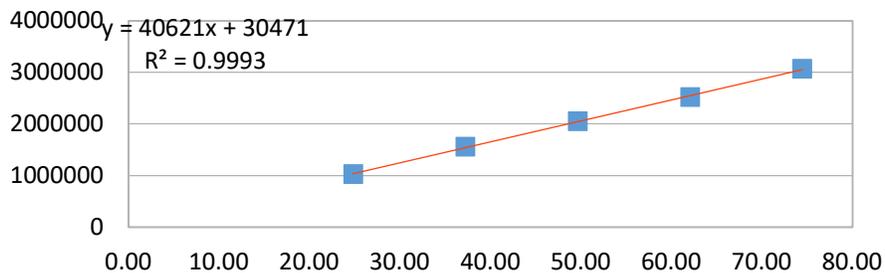**Figure 22: Calibration curve of Amlodipine Besylate**

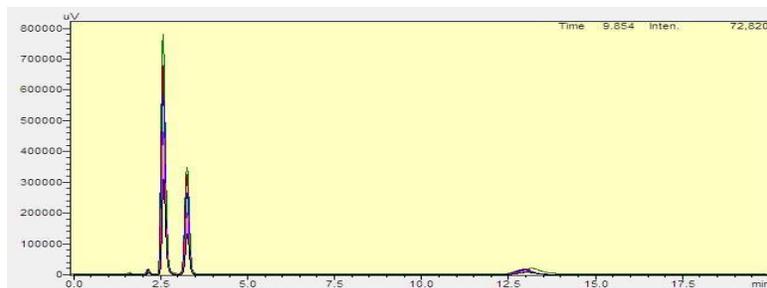**Figure 23: Calibration curve of Chlorthalidone**

Figure 24: Overly Chromatogram of different concentration of mixtures of Metoprolol tartrate, Amlodipine Besylate and Chlorthalidone
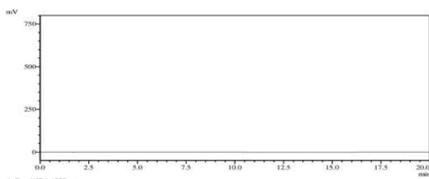
Specificity

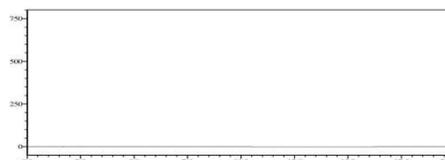**Figure 25: Chromatogram of Diluent for specificity**

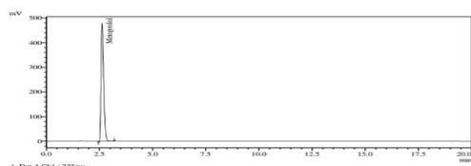**Figure 26: Chromatogram of Placebo**

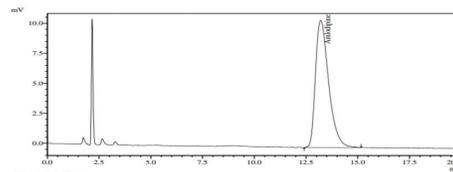**Figure 27: Chromatogram of Metoprolol tartrate specificity**

Figure 28: Chromatogram of Amlodipine Besylate
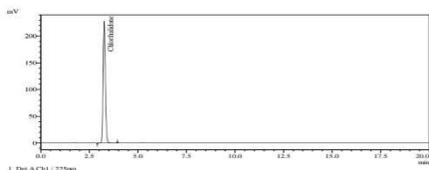

Figure 29: Chromatogram of Chlorthalidone specificity
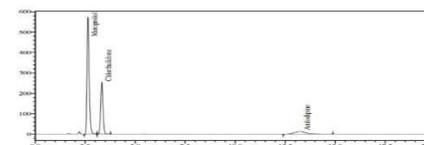
Figure 30: Chromatogram of Sample specificity
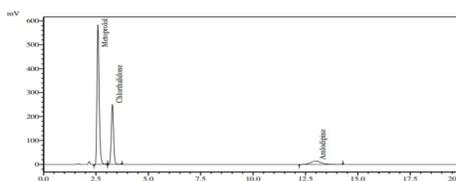
Figure 31: Chromatogram of Standard specificity

Table 12: System Suitability Study Data

Sr. No.	Area of Metoprolol Tartrate	Area of Amlodipine Besylate	Area of Chlorthalidone
1	4375433	507136	1976425
2	4360936	507085	1984681
3	4340303	503622	1970746
4	4346328	507094	1975269
5	4337799	503172	1975785
Mean	4352160	505622	1976581
SD	15807.35	2037.26	5050.65
%RSD	0.4	0.4	0.3
Retention Time	1.44	1.33	1.13
Theoretical Plates	2899	3685	4347
Tailing Factor	2.74	13.447	3.491

Table 13: Accuracy Data for Metoprolol Tartrate

Conc. Level	Set No.	Amount Added (mg)	Amount Recovered (mg)	% Recovery	Mean % Recovery	SD	% RSD
50%	1	0.48	0.48	101.6	101.7	0.17	0.2
	2	0.48	0.48	101.6			
	3	0.48	0.49	101.9			
100%	1	0.95	0.94	98.5	99.0	0.80	0.8
	2	0.95	0.94	98.5			
	3	0.95	0.95	99.9			
150%	1	1.43	1.43	100.3	100.5	1.21	1.2
	2	1.43	1.46	101.8			
	3	1.43	1.42	99.4			

Table 14: Accuracy Data for Amlodipine Besylate

Conc. Level	Set No.	Amount Added (mg)	Amount Recovered (mg)	% Recovery	Mean % Recovery	SD	% RSD
50%	1	0.35	0.35	98.3	98.4	0.23	0.2
	2	0.35	0.35	98.7			
	3	0.35	0.35	98.3			
100%	1	0.71	0.70	99.8	100.4	0.65	0.6
	2	0.71	0.71	100.4			
	3	0.71	0.71	101.1			
150%	1	1.06	1.07	101.2	101.3	0.23	0.2
	2	1.06	1.07	101.6			
	3	1.06	1.07	101.2			

Table 15: Accuracy Data for Chlorthalidone

Conc. Level	Set No.	Amount Added (mg)	Amount Recovered (mg)	% Recovery	Mean % Recovery	SD	% RSD
50%	1	0.23	0.23	100.5	100.7	0.20	0.2
	2	0.23	0.23	100.9			
	3	0.23	0.23	100.6			
100%	1	0.46	0.46	99.7	100.1	0.69	0.7
	2	0.46	0.46	99.7			
	3	0.46	0.46	100.9			
150%	1	0.69	0.69	100.1	99.8	0.46	0.5
	2	0.69	0.69	100.1			
	3	0.69	0.68	99.3			

Table 16: Repeatability data for Metoprolol Tartrate, Amlodipine Besylate and Chlorthalidone

Metoprolol Tartrate		Amlodipine Besylate		Chlorthalidone	
Conc. (µg/ml)	Area (n=6)	Conc. (µg/ml)	Area (n=6)	Conc. (µg/ml)	Area (n=6)
100%	4427421	100%	531355	100%	2095831
	4374235		531327		2070593
	4416060		532295		2088539
	4371102		531068		2066720
	4348813		528795		2051516
	4438439		534021		2090609
Mean	4396012	Mean	531477	Mean	2077301
SD	36085.97	SD	1705.21	SD	17138.12
% RSD	0.8	% RSD	0.3	% RSD	0.8

Table 17: Intermediate Precision for Metoprolol Tartrate

Conc. (µg/ml)	Area (n=3)	Mean	SD	% RSD
50%	2216602	2229970	24055.08	1.0
	2257740			
	2215568			
100%	4339019	4380424	39489.62	0.9
	4417669			
	4384583			
150%	6829354	6771135	54336.95	0.8
	6762283			
	6721767			

Table 18: Intermediate Precision data for Amlodipine Besylate

Conc. (µg/ml)	Area (n=3)	Mean	SD	% RSD
50%	260148	260998	924.38	0.3
	261982			
	260863			
100%	525538	530990	5460.52	1.0
	536459			
	530972			
150%	802710	802063	5740.38	0.7
	796027			
	807453			

Table 19: Intermediate Precision data for Chlorthalidone

Conc. (µg/ml)	Area (n=3)	Mean	SD	% RSD
50%	1027653	1031808	7535.18	0.7
	1040506			
	1027265			
100%	2050153	2069944	18917.36	0.9
	2087846			
	2071833			
150%	3189650	3159340	27567.41	0.8
	3152609			
	3135762			

Table 20: Limit of Detection data for Metoprolol Tartrate, Amlodipine Besylate and Chlorthalidone

Metoprolol Tartrate	Amlodipine Besylate	Chlorthalidone
LOD = 3.3 * σ/S	LOD = 3.3 * σ/S	LOD = 3.3 * σ/S
$= \dfrac{3.3 \times 6042.5712}{8225.08}$	$= \dfrac{3.3 \times 5310.259}{10012.56}$	$= \dfrac{3.3 \times 23775.77}{40621.08}$
= 2.424 µg/ml	= 1.750 µg/ml	=1.931 µg/ml

Table 21: Limit of Quantitation data for Metoprolol Tartrate, Amlodipine Besylate and Chlorthalidone

Metoprolol Tartrate	Amlodipine Besylate	Chlorthalidone
LOQ = 10 * σ/S	LOQ = 10 * σ/S	LOQ = 10 * σ/S
$= \dfrac{10 \times 6042.5712}{8225.08}$	$= \dfrac{10 \times 5310.259}{10012.56}$	$= \dfrac{10 \times 23775.77}{40621.08}$
= 7.347 µg/ml	= 5.304 µg/ml	=5.853 µg/ml

Table 22: Robustness Data of Metoprolol Tartrate

Sr. No.	Area at Temp. -5°C	Area at Temp. +5°C	Area at Flow Rate -10%	Area at Flow Rate +10%	Area at Organic Phase -2%	Area at Organic Phase +2%
1	4208953	4206014	4663966	3980834	4469023	3688364
2	4225319	4212728	4667582	3984816	4474126	3691786
3	4220973	4208812	4669559	3994641	4468140	3692613
Mean	4218415	4209185	4667036	3986764	4470430	3690921
SD	8477.55	3372.47	2836.24	7106.57	3231.42	2252.70
% RSD	0.2	0.1	O.1	0.2	0.1	0.1
Theoretical Plates	3070	3173	3164	3012	3108	3074
Tailing Factor	1.45	1.46	1.43	1.41	1.45	1.48
Retention Time	2.66	2.55	2.85	2.485	2.59	2.55

Table 23: Robustness Data of Amlodipine Besylate

Sr. No.	Area at Temp. -5°C	Area at Temp. +5°C	Area at Flow Rate -10%	Area at Flow Rate +10%	Area at Organic Phase -2%	Area at Organic Phase +2%
1	4208953	4206014	4663966	3980834	4469023	3688364
2	4225319	4212728	4667582	3984816	4474126	3691786
3	4220973	4208812	4669559	3994641	4468140	3692613
Mean	4218415	4209185	4667036	3986764	4470430	3690921
SD	1281.86	544.45	625.32	970.77	903.32	2682.34
% RSD	0.2	0.1	0.1	0.2	0.1	0.1
Theoretical Plates	3070	3173	3164	3012	3108	3074
Tailing Factor	1.45	1.46	1.43	1.41	1.45	1.48
Retention Time	15.128	12.502	14.546	12.005	13.651	13.128

Table 24: Robustness Data of Chlorthalidone

Sr. No.	Area at Temp. -5°C	Area at Temp. +5°C	Area at Flow Rate -10%	Area at Flow Rate +10%	Area at Organic Phase -2%	Area at Organic Phase +2%
1	1912757	1914372	2118694	1800154	2130930	1809849
2	1919644	1917657	2121240	1804285	2135094	1813812
3	1918577	1915834	2121882	1805020	2131400	1814333
Mean	1916993	1915954	2120605	1803153	2132475	1812665
SD	3706.78	1645.80	1686.10	2623.08	2280.54	2452.31
% RSD	0.2	0.1	0.1	0.1	0.1	0.1
Theoretical Plates	4021	4205	4868	4176	4083	4050
Tailing Factor	1.20	1.22	1.20	1.20	1.21	1.22
Retention Time	3.42	3.15	3.583	3.08	3.28	3.19

Table 25: Assay of Metoprolol Tartrate

Sr. No. (n=3)	Area of Sample	% Assay	Mean % Assay	SD	% RSD
1	2216602	100.1	100.7	24055.08	1.1
2	2257740	102.0			
3	2215568	100.1			

Table 26: Assay of Amlodipine Besylate

Sr. No. (n=3)	Area of Sample	% Assay	Mean % Assay	SD	% RSD
1	260148	98.6	98.9	924.38	0.3
2	261982	99.2			
3	260863	98.8			

Table 27: Assay of Chlorthalidone

Sr. No. (n=3)	Area of Sample	% Assay	Mean % Assay	SD	% RSD
1	1027653	103.2	103.6	7535.18	0.7
2	1040506	104.4			
3	1027265	103.1			

Table 25: Summary of Validation Parameters of Metoprolol Tartrate, Amlodipine Besylate and Chlorthalidone

Parameter		Metoprolol Tartrate	Amlodipine Besylate	Chlorthalidone
Specificity		Specific		
Linearity and Range		245.53-736.60	25.06-75.18	24.81-74.44
Correlation Co-efficient (R^2)		0.99866	0.99864	0.99934
Precision (%RSD)	Method Precision (Repeatability)	0.8	0.3	0.8
	Intermediate Precision	1.0-0.8	0.3-0.7	0.7-0.8
Accuracy (% Recovery)	50%	101.7	98.4	100.7
	100%	99.0	100.4	100.1
	150%	100.5	101.3	99.8
Limit of Detection (LOD)		2.424 µg/ml	1.750 µg/ml	1.931 µg/ml
Limit of Quantitation (LOQ)		7.347 µg/ml	5.304 µg/ml	5.853 µg/ml
Robustness		The system suitability parameters were found well within the acceptance criteria as per system suitability.		
% Assay		100.7%	98.9%	103.6%

CONCLUSION:

Initial identification tests for the received APIs of Metoprolol Succinate, Telmisartan, and Amlodipine Besylate were successful in confirming their identities. Metoprolol Tartrate, Amlodipine Besylate and Chlorthalidone were simultaneously estimated using a novel RP-HPLC approach. With a stationary phase of column Cosmosil C_{18} (250 mm × 4.6 mm internal diameter × 5 micron particle size). Mobile phase of Phosphate Buffer pH 2.5: ACN: Methanol in the ration of (50:30:20%v/v), at a flow rate of 1.5 mL/min. Temperature was fixed at 25°C, and the detection wavelength was held constant at 222 nm. Retention times for Metoprolol Tartrate, Amlodipine Besylate and Chlorthalidone were found to be 1.44, 1.33 and 1.13 respectively. The Proposed method can be utilized for the routine analysis of Metoprolol Tartrate, Amlodipine Besylate and Chlorthalidone. Linearity range of 245.53-736.60, 25.06-75.18, and 24.81-74.44 µg/ml. The proposed technique is new, simple, precise, linear, sensitive, robust, and accurate for simultaneous estimation of Metoprolol Succinate, Telmisartan, and Amlodipine Besylate in Synthetic Mixture,

according to the results of the experiment. For routine analysis, this approach was more stable and cost-effective.

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