Available online on 18.08.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Comparative Study of Antihypertensive Drugs Amlodipine Besylate /Metoprolol Succinate and Nebivolol Hydrochloride /Valsartan Combinations in Bilayer Tablets

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ABSTRACT

The present research is an approach to develop a formulation platform that shall help in minimizing the time and effort taken to develop a drug delivery system. Taking bilayer tablet technology as a representation for drug delivery system, well accepted antihypertensive drugs, Amlodipine besylate and Metoprolol succinate were considered as model drugs for the study. Initially the process variables like concentration of the disintegrants, Sodium starch Glycolate and cross carmellose sodium, Polymers HPMC K100M and K4M were standardized with these drugs so that the incorporation of a new combination drugs would provide predictable results with a minimal trial runs. Nebivolol hydrochloride and Valsartan were considered as test drugs since they are novel antihypertensive drug combination and their physicochemical and pharmacokinetic parameters were almost similar to that of the model drugs. The r value 0.98943 indicates a good correlation between the release profile of Amlodipine besylate (model drug) and Nebivolol hydrochloride (test drug) from the IR layer. Similarly, the r value in the range of 0.9998 indicates a good correlation between the release profile of Metoprolol succinate (model drug) and Valsartan (test drug) from the SR layer. The comparable experimental results of the model drugs and test drugs considered for this study infer that if two drugs are similar in their physicochemical and pharmacokinetic parameters, their behavior with respect to in vitro parameters will be similar provided formulation variables remains constant. This concept could be productive in developing drug delivery system for new drugs for which extensive research and time are major constraints.

Keywords: Bilayer tablets, fixed unit dosage form, Amlodipine besylate, Metoprolol Succinate Nebivolol hydrochloride, Valsartan.

Article Info: Received 06 June 2019; Review Completed 23 July 2019; Accepted 04 Aug 2019; Available online 18 August 2019

Cite this article as:

Arunkumar S, Srinivas L, Satyavati D, Emmanuel C, A Comparative Study of Antihypertensive Drugs Amlodipine Besylate /Metoprolol Succinate and Nebivolol Hydrochloride /Valsartan Combinations in Bilayer Tablets, Journal of Drug Delivery and Therapeutics. 2019; 9(4-s):529-539 http://dx.doi.org/10.22270/jddt.v9i4-s.3214

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INTRODUCTION

Developing a useful drug delivery system is a result of concerned efforts by the scientist of variety of disciplines who recognize the need and potential for improving pharmcotherapeutics through the development of novel drug delivery systems. The method by which a drug is delivered can have a significant effect on its efficacy. Some drugs have an optimum concentration range within which maximum benefit is derived, and concentrations above or below this range can be toxic or produce no therapeutic benefit at all. In recent times, many guidelines emphasize that the majority of the hypertensive population will require two or more antihypertensive drugs to achieve the recommended treatment goals.[1]

Fixed drug combinations (FDCs) of antihypertensive agents have proven to be efficacious in the treatment of hypertension. The availability of many antihypertensive agents in various classes such as diuretics, calcium channel blockers (CCBs), angiotensin converting enzyme inhibitors, angiotensin receptor blockers, β adrenoceptor blockers are frequently combined in FDCs.

A combination of a calcium channel blocker (CCB) Amlodipine besylate with a β adrenoceptor blocker Metoprolol succinate is advantageous since, their modes of action are different yet their action on blood pressure (BP) is complementary. The β adrenoceptor blocker might regulate any CCB-induced acute reflex increase in sympathetic activity and conversely, the CCB might compensate the peripheral vasospasm and drop in

cardiac output caused by the β adrenoceptor blocker; thus, reducing the overall burden of side-effects. This is one key to ensure better long-term compliance with therapy and to more effective long-term BP control.[2]

Metoprolol succinate and Amlodipine Besylate are in the market for the past two decades or so. These drugs have been extensively researched in various dimensions for optimal hypertension therapy. Hence they were considered as model drugs to develop bilayer tablets which shall help in developing bilayer tablets of recently approved fixed dose combination of Nebivolol Hydrochloride and Valsartan for hypertension.

MATERIALS AND METHODS

Metoprolol succinate, Amlodipine Besylate, Nebivolol hydrochloride and Valsartan were received as gift samples from Apotex Pharmachem India Pvt. Ltd, Bengaluru, India. The polymers such as Hydroxypropyl Methylcellulose K100M (Methocel K100M premium), K4M (Methocel K4M premium), Dibasic calcium phosphate (anhydrous, FCC), sodium starch glycolate (731713H), croscarmellose sodium(Ac di sol NF), FD&C blue lake, microcrystalline cellulose, silicon dioxide (Aerosil 200) and magnesium stearate (ligamed MF) were obtained from KMS Pharma - Formulation development Healthcare Pvt Ltd, Chennai, India.

The present research was carried out in two phases. The phase I consisted of formulation and evaluation of bilayer tablets of model drugs Amlodipine besylate and Metoprolol succinate for immediate release (IR) and sustained (SR) respectively. The II phase consisted of formulation and evaluation of bilayer tablets of test drugs Nebivolol hydrochloride and Valsartan in IR and SR respectively.

Phase I: Bilayer tablets of Amlodipine besylate and Metoprolol succinate

Selection of excipients

The excipients necessary for formulation was selected based on the relevant information from the literature survey on previous research on the same ideology. The excipients which influence modified release in the bilayer tablets such as superdisintegrants: Sodium Starch Glycolate, Croscarmellose sodium and the percentage of polymers like Hydroxypropyl Methylcellulose K100M, K4M were optimized by reference articles.[3] The selected excipients were subjected for incompatibility studies with the drugs.

The ingredients after sifting, prelubrication and blending was subjected for precompression parameters like excipient compatibility studies, Angle of Repose, Bulk Density, Tapped Density Carr's. Index (%), Hausner's Ratio.[4] (Table 1) the Fourier-transform infrared spectroscopy (FTIR) studies were done for standard Amlodipine Besylate, Metoprolol succinate and Excipients.

Table 1: Pre-compression Parameters of immediate and sustained release layers

Formulation	Angle of Repose*	Bulk Density* (g/ml)	Tapped Density* (g/ml)	Carr's Index * (%)	Hausner's Ratio*
F1	27.1°±0.03	0.365±0.0006	0.581± 0.0021	37.17± 0.74	1.591± 0.009
F2	28°±0.32'	0.362±0.0007	0.579± 0.0023	37.47± 0.76	1.599± 0.007
F3	29°±0 32'	0.360±0.0005	0.584± 0.0018	38.35± 0.75	1.622± 0.005
F4	28°±035'	0.362±0.0007	0.580± 0.0019	37.58± 0.78	1.580± 0.006
F5	28°±045'	0.364±0.0081	0.582± 0.0022	37.45± 0.79	1.598± 0.009
F6	28°±053'	0.370±0.0082	0.578±0.0024	37.98± 0.74	1.562± 0.004
F7	28⁰±010'	0.340+0.0073	0.548+ 0.0037	37.65+ 0.79	1.611+ 0.006
F8	29⁰±033'	0.351+0.0008	0.568+ 0.0036	38.20+ 0.78	1.618+ 0.008
F9	28⁰±065'	0.372+0.0091	0.592+ 0.0041	37.16+ 0.76	1.591+ 0.089
F10	29⁰±044'	0.365+0.0092	0.587+ 0.0042	37.81+ 0.75	1.608+ 0.091

Where, *All values are mean ± SD, n=3,

Formulation of Bi-layer tablets:

Bi-layer tablets of extended release Metoprolol succinate and immediate release Amlodipine Besylate were prepared through direct compression method according to the composition shown in Table 2. Various step like sifting, dry mixing, prelubrication and lubrication was carried out prior direct compression process.

Preparation of Amlodipine Besylate immediate release (IR) layer:

Amlodipine Besylate immediate release tablets were prepared by using direct compression method. The microcrystalline cellulose, Dicalcium phosphate, Pregelatinised starch, sodium starch gluconate and the active ingredient were passed through sieve no. 30 and mixed homogenously. Magnesium stearate and Aerosil were passed through sieve no.60 and added as a lubricant to the above dry mix and mixed well for 5 minutes. Finally the colorant FD &C blue lake was sieved through sieve no.100 mesh and then mixed with the dry mix homogenously to get uniform blend without mottling.

Preparation of Metoprolol sustained release (SR) layer:

The active ingredient Metoprolol succinate, was passed through the 40 mesh sieve followed by the other ingredients. Metoprolol succinate, Polymer [Hydroxypropyl Methylcellulose K100M (Methocel K100M premium), K4M (Methocel K4M premium)] diluents [Dibasic calcium Phosphate (anhydrous, FCC)] were taken in a planetary mixer and mixed for 15 minutes to ensure uniform mixing of the ingredients with the drug.Colloidal slicondioxide (Aerosil-200), sifted through 40 mesh sieve were mixed with dry mixed blend for 5 minutes. [5]

1. Tablet compression:

The Bi-layer tablet compression was made using 14/6mm punches in Rimek minicompressor II DL. In this, sustained release Metoprolol Succinate powder were introduced first in to the die cavity and a slight precompression was made so that the layer was uniformly distributed. After that immediate release Amlodipine Besylate granules were added through the other feed and a final compression was made with view to maintain the fixed hardness and uniform weight.

Table 2: Composition of different formulations

S.No	Ingredients	F1		F2		F3		F4		F5		F6		F7		F8		F9		F10	
		mg/Tab	%W/W	mg/Tab	%W/W	mg/Tab	%W/W	mg/Tab	%W/W	mg/Tab	%W/W	mg/Tab	%W/W	mg/Tab	%W/W	mg/Tab	%W/W	mg/Tab	%W/W	mg/Tab	%W/W
First layer																					
1	Amlodipine Besylate	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8
2	Microcrystalline cellulose (Avicel PH 102)	37.95	37.95	32.95	32.95	34.95	34.95	34.95	34.95	32.95	32.95	37.95	37.95	37.95	37.95	37.95	37.95	37.95	37.95	37.95	37.95
3	Dibasic calcium Phosphate	50	50	55	55	55	55	55	55	55	55	50	50	52	52	52	52	50	50	50	50
4	Sodium starch Glycolate	4	4	4	4	2	2	0	0	0	0	0	0	2	2	2	2	0	0	0	0
5	Croscarmellose sodium	0	0	0	0	0	0	2	2	4	4	4	4	0	0	0	0	4	4	4	4
6	FD&C blue lake	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
7	Mg. Stearate	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Total		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Second layer																					
8	Metoprolol Succinate	57.5	28.75	57.5	28.75	57.5	28.75	57.5	28.75	57.5	28.75	57.5	28.75	57.5	28.75	57.5	28.75	57.5	28.75	57.75	28.75
9	Microcrystalline cellulose (Avicel PH 102)	88.5	44.25	100.5	50.25	94.5	47.25	94.5	47.25	78.5	39.25	58.5	29.25	38.5	19.25	28.5	14.25	58.5	29.25	18.5	9.25

Table 3: Physical characterizations of Bi-layer tablets

Batch code	Avg weight mg	Hardness* kg	Thickness* mm	Drug content (%)		Friability (%)
				Amlodipine	Metoprolol	
F1	285	10.3± 0.1	3.52± 0.024	94.1	91.6	0.54
F2	299	10.8±0.07	3.63± 0.07	91.7	100.7	0.57
F3	302	12.4± 0.084	3.64± 0.033	93.1	97.5	0.49
F4	296	11.4± 0.1	3.62±0.055	92.1	91.5	0.56
F5	294	12.2±0.063	3.59+ 0.07	93.3	96.6	0.52
F6	305	11.7±0.1	3.77±0.1	90.9	99.2	0.49
F7	302	12.4±0.16	3.64± 0.16	93.1	97.5	0.49
F8	299	10.8+ 0.14	3.63± 0.08	91.7	100.7	0.57
F9	305	11.7+ 0.09	3.77+ 0.07	90.9	99.2	0.49
F10	294	12.2+ 0.14	3.59+ 0.12	93.3	96.6	0.52

Where, *All values are mean ± SD, n=3,

2. In vitro dissolution study [6]

Drug Release Studies for Immediate release (IR) layer

The in vitro dissolution of immediate release layer was determined using USP XXIII (basket method) dissolution apparatus. The basket was allowed to rotate at a speed of 100 rpm and temperature of 37 ± 0.5°C was maintained. The dissolution medium used was 900 ml of 0.1N HCl (pH 1.2) for 2 hours. Aliquots (5 ml) of sample were collected at 30 min from the dissolution apparatus and it was replaced with equal volume of fresh dissolution medium. The aliquots withdrawn were filtered through 0.45µm millipore filters. The concentration of Amlodipine in the dissolution media was estimated by HPLC method at 239 nm.

Drug Release Studies for Metoprolol Succinate sustained release (SR) layer[7]

The *in vitro* dissolution of sustained release layer was determined using USP XXIII (basket method) dissolution apparatus. The basket was allowed to rotate at a speed of 100 rpm and temperature of 37 ± 0.5°C was maintained. The dissolution medium used was 900 ml of 0.1N HCl (pH 1.2) for the initial 2hours followed by study in simulated intestinal fluid Phosphate buffer solution (pH 6.8). Aliquots (5 ml) of

sample were collected at predetermined time intervals (1, 4, 8, 20 hrs) from the dissolution apparatus and it was replaced with equal volume of fresh dissolution medium. The aliquots withdrawn were filtered through 0.45µm millipore filters. The concentration of Metoprolol in the dissolution media was estimated by HPLC method at 280 nm.

Drug Release Studies for Bi-layer Tablets

The *in vitro* dissolution of Amlodipine and Metoprolol Bi-layer tablets were determined using USP XXIII (basket method) dissolution apparatus. The basket was allowed to rotate at a speed of 100 rpm and temperature of 37 ± 0.5°C was maintained. The dissolution medium used was 900 ml of 0.1N HCl (pH 1.2) for the initial 2hours followed by study in simulated intestinal fluid Phosphate buffer solution (pH 6.8). Aliquots (5 ml) of sample were collected at predetermined time intervals (1, 4, 8, 20 hrs) from the dissolution apparatus and it was replaced with equal volume of fresh dissolution medium. The aliquots withdrawn were filtered through 0.45µm millipore filters. The concentration of both the drugs in the dissolution media was estimated by HPLC method at 239 nm and 280nm for Amlodipine and Metoprolol respectively.

Table 4: Dissolution parameters of Amlodipine IR and Metoprolol ER tablets

Particulars	Amlodipine	Metoprolol
Dissolution media	0.01N HCl	pH 6.80 Phosphate Buffer
Volume	500ml	500ml
Model	USP TYPE II Paddle (covered with Parafilm)	USP TYPE II Paddle (covered with Parafilm)
RPM	75	50
Time of sampling	30mins	1,4,8,20 Hrs

Table 5: Dissolution profile for Amlodipine Besylate layer

Time (min)	F1*	F2*	F3*	F4*	F5*	F6*	F7*	F8*	F9	F10
30	% drug released									
	100 ±5.08	108.2 ±4.6	103.2 ±3.53	107.4 ±1.48	105.5 ±1.5	102.2 ±1.94	65.4 ±1.54	74.9 ±0.62	95.9 ±0.5	96 ± 0.6

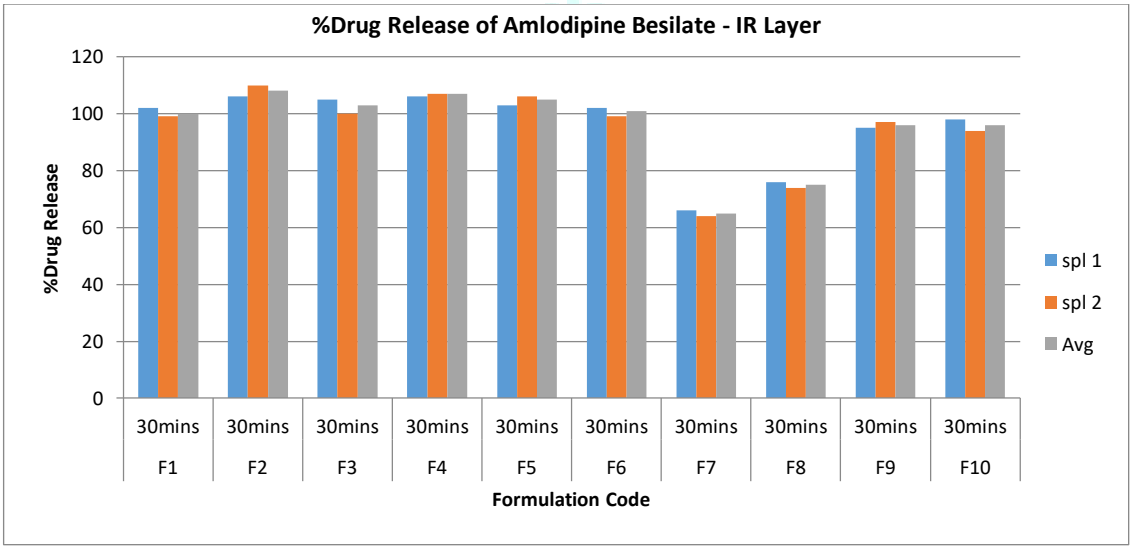

Figure 1: Drug Release from Amlodipine Besylate IR Layer (spl –trial 1, 2 respectively)

Table6: Drug Release from Metoprolol Succinate sustained release (SR) layer

Time in hours	limit of %drug release	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	MKT PD*
1	Not more than 25%	17.0 +3.70	13.0 +4.95	12.6 ±4.83	17.1 ±4.10	21.4 ±5.53	22.0 ±5.10	30.0 ±4.95	27.6 ±4.83	19.4 ±5.26	14.3 ±4.66	19.1±5.30
4	Between 20% and 40%	31.0 ±2.53	24.4 ±4.56	25.2 ±3.79	29.5 ±2.96	31.8 ±2.40	36.6 ±4.38	56.4 ±4.56	50.2 ±3.79	40.2 ±5.80	33.8 ±4.35	32.5±4.43
8	Between 40% and 60%	51.2 ±2.25	47.1 ±2.84	49.6 ±3.73	47.2 ±3.43	49.7 ±2.81	59.5 ±3.33	75.1 ±2.84	70.6 ±3.73	62.1 ±1.78	51.9 ±3.52	54.6 ±4.75
20	Not less than 80%	98.4 ±1.83	99.7 ±1.67	99.5 ±1.42	100 ±1.63	96.1 ±0.70	98.4 ±0.88	97.7 ±1.67	97.5 ±1.42	97.9 ±0.98	96.5 ±3.28	99.6 ±1.02

MKT PD: Marketed product

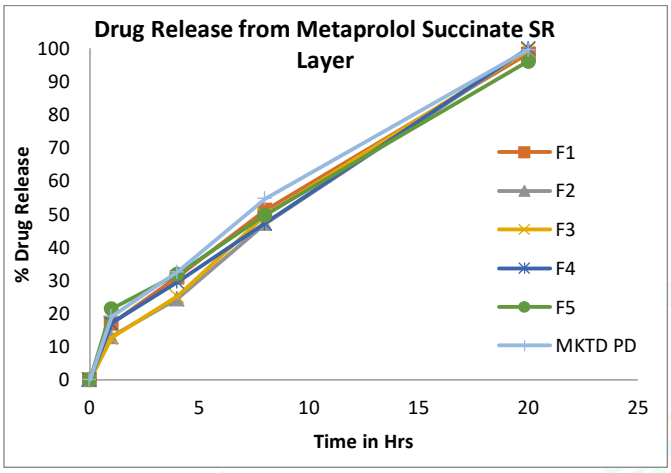

Figure 2: Drug Release from Metoprolol Succinate SR layer for formulations 1 to 5

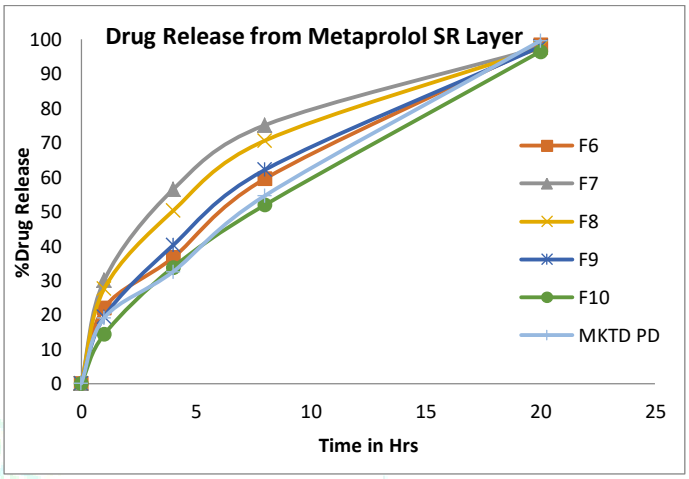

Figure 2A: Drug Release from Metoprolol SR Layer for formulations 6 to 10

Table 7: Release kinetics

	Model			
	Formulation code	Zero order	Higuchi	Korsemeyer peppas
Correlation coefficient	F1	0.99962	0.98728	0.81490
	F2	0.99555	0.97312	0.85230
	F3	0.99415	0.97665	0.85596
	F4	0.99181	0.97914	0.81456
	F5	0.98212	0.98677	0.78028
	F10	0.98395	0.99187	0.83323

Interpretation of the kinetic studies:

- The r value for zero order release indicates the SR layer containing valsartan follows zero order release pattern where in the drug release at any moment is independent of the initial concentration.
- The r value for Higuchi model infers the SR layer follows a drug release by diffusion process based on Fickian law of diffusion.
- The r value for Peppas model shows moderate linearity.

Phase II: Bilayer tablets of Nebivolol Hydrochloride and Valsartan

In the best formulation selected from Phase I experiments, Amlodipine besylate was replaced with Nebivolol hydrochloride and Metoprolol succinate was replaced with Valsartan. These drugs were considered as main drugs since the combination is novel and has many therapeutic benefits.[9]

Rationale for drug selection

Recently, fixed dose combination of Nebivolol hydrochloride, a selective β1 antagonist and valsartan an angiotensin II receptor blocker was approved by USFDA for hypertension. Pharmacological profiles of Nebivolol hydrochloride and valsartan alone and in combination are well characterized. In addition, a large 8-week randomized trial in stages I–II hypertensive patients (N=4161) demonstrated greater blood pressure-reducing efficacy for 33Neb/valsartan SPCs than component monotherapies with comparable tolerability. In a biomarkers sub study (N=805), Nebivolol/valsartan single-pill combination prevented valsartan-induced increases in plasma renin, and a greater reduction in plasma aldosterone was observed with the highest single-pill combination dose vs. valsartan 320 mg/day.[10]

Nebivolol, a new anti-hypertensive drug with peripheral vasodilating properties and adrenoceptor antagonism, given in monotherapy, is as effective as amlodipine in reducing clinical BP in elderly patients with essential hypertension, but it is better tolerated and has the additional advantage of reducing sympathetic discharge. Nebivolol may therefore be recommended as a first-line treatment option for the management of elderly patients with mild to moderate uncomplicated essential hypertension.[11]

Valsartan is an angiotensin II receptor antagonist that is used in the treatment of hypertension. It act by blocking the binding of angiotensin II and I to its receptors thereby blocking vasoconstrictor and aldosterone secreting effect of angiotensin II selectively. The most preferred route for this drug is oral delivery in the form of tablets. Valsartan has poor water solubility, low bioavailability (approximately 20-25%) and short half-life (nearly hrs.) which makes it an ideal candidate for sustained release. Hence in the present work this ideology is adopted.[12]

A comparative physicochemical properties of the four drugs in study has been summarized in table 8. The values show that the model drugs and the test drugs are comparable in their physicochemical and pharmacokinetic properties thus justifying the selection and replacement of the model drugs in the phase I study with the test drugs. Further, similarities in the behavior of the test drugs to the model drugs with respect to the precompression and post compression parameters can be understood by formulating the test drugs and evaluating them in same manner as that of the model drugs.

Table 8: Comparative physicochemical parameter of the drugs[13, 14, 15, 16, 17, 18, 19]

Parameter	Drugs for immediate release		Drugs for sustained release	
	Amlodipine besylate (model drug)	Nebivolol hydrochloride(test drug)	Metoprolol succinate (model drug)	Valsartan(test drug)
Solubility in pH 1.2/ 6.8*	Highly soluble	Highly soluble	Good solubility	Low solubility
Solubility in pH 6.8*	Less soluble	Less soluble	Highly soluble	Highly soluble
Lipid solubility	Highly soluble	Highly soluble	Highly soluble	Highly soluble
Existence	Weak acid	Weak acid	Weak base	acidic
pKa	8.60	8.13	9.6	8.15
Permeability	Highly permeable	Highly permeable	Highly permeable	Low permeable in pH. 1.2, highly permeable in pH 6.8
Bioavailability	64-90%	12-96%	50%	
Protein binding	93%	98%	12%	94 to 97%
t half	30-40 hrs	10 to 31hrs	3-7h	4-6 hr.
Tmax	3-8hrs	0.5-4hrs	1.5 – 2.0 h	1.0 – 2.0 hr.
Volume of distribution	21 L/kg	17.3-184L/kg	3.2 - 5.6 L/kg.	17 L
clearance	21h	16-657L/h	1 L/minute.	0.62 L/hr
Partition coefficient	2.66	4.03	1.57	0.033

Formulation of Bi-layer tablets:

The ingredients after sifting, Prelubrication and blending was subjected for precompression parameters like excipient compatibility studies, Angle of Repose, Bulk Density, Tapped Density Carr's. Index (%), Hausner's Ratio.[20] The results are shown in table 9 and 10. The studies were done for standard Nebivolol HCl, Valsartan and Excipients.

Table 9: Pre-compression Parameters of Nebivolol hydrochloride

Formulation	Angle of Repose*	Bulk Density*	Tapped Density*	Carr's. Index (%)*	Hausner's Ratio*
B1	28°.46'±0.26	0.6130±0.0071	0.799±0.0021	23.28±0.71	1.304±0.008
B2	28°.46'±0.35	0.613±0.0071	0.799±0.0024	23.28±0.75	1.304±0.007
B3	28°.46'±0.72	0.613±0.091	0.799v0.0019	23.28±0.80	1.304±0.098
B4	27°.23'±0.27	0.597±0.0091	0.789±0.0030	23.94±0.69	1.315±0.009
B5	27°.23'±0.34	0.597±0.0092	0.789±0.0023	23.94±0.73	1.315±0.089
B6	27°.23±0.70	0.597±0.0074	0.789±0.0018	23.94±0.79	1.315±0.097

Where, *All values are mean ± SD, n=3,

Table10: Pre-compression Parameters of Valsartan

Formulation	Angle of Repose*	Bulk Density*	Tapped Density*	C. Index (%)*	Hausner's Ratio*
B1	27^0.28'±0.27	0.329±0.0094	0.5280±0.0019	37.73±0.77	1.606±0.007
B2	28^0.10'±0.71	0.335±0.0075	0.533±0.0025	37.25±0.74	1.594±0.006
B3	28^0.35'±0.77	0.305±0.008	0.536±0.0028	38.137±0.81	1.759±0.097
B4	28^0.32'±0.49	0.350±0.009	0.534±0.0026	34.42±0.79	1.525±0.078
B5	28^0.36'±0.25	0.333±0.0069	0.522±0.0024	36.26±0.82	1.569±0.008
B6	28^0.56'±0.78	0.306±0.0077	0.486±0.0029	36.98±0.74	1.587±0.087

Where, *All values are mean ± SD, n=3,

Six formulations of bilayer tablets were prepared with the variation of excipients as mentioned Table 11. Various steps (Sifting, Dry mixing, Prelubrication and Lubrication) involved in direct compression process. The formulation procedure was same as the formulation of model drugs. The compressed tablets were analyzed for post compression parameters. The details of the results are shown in Table 12.

Table 11: Composition of Nebivolol Hydrochloride and Valsartan in IR layer and SR layer

Nebivolol Hydrochloride and Valsartan Bilayer tablets												
Formulation Plan												
S.No	Ingredients	B1		B2		B3		B4		B5		B6	
		mg/Tab	%W/W	mg/Tab	%W/W	mg/Tab	%W/W	mg/Tab	%W/W	mg/Tab	%W/W	mg/Tab	%W/W
First layer													
1	Nebivolol Hydrochloride	5	5	5	5	5	5	5	5	5	5	5	5
2	Microcrystalline cellulose (Avicel PH 102)	37.95	37.95	32.95	32.95	34.95	34.95	34.95	34.95	32.95	32.95	37.95	37.95
3	Dibasic calcium Phosphate	51.8	51.8	51.8	51.8	51.8	51.8	51.8	51.8	51.8	51.8	51.8	51.8
4	Sodium starch Glycolate	4	4	4	4	4	4	0	0	0	0	0	0
5	Croscarmellose sodium	0	0	0	0	0	0	4	4	4	4	4	4
6	FD&C blue lake	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
7	Mg. Stearate	1	1	1	1	1	1	1	1	1	1	1	1
Total		**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Second layer													
8	valsartan	60	23.75	60	23.75	60	23.75	60	23.75	60	23.75	60	23.75
9	Microcrystalline cellulose (Avicel PH 102)	86	49.25	98	55.25	91	52.25	92	52.25	76	44.25	16	34.25
10	HPMC K100M	50	25	38	19	44	22	0	0	0	0	0	0
11	HPMC K4M	0	0	0	0	0	0	44	22	60	30	120	40
12	Sio2	2	1	2	1	2	1	2	1	2	1	2	1
13	Mg. Stearate	2	1	2	1	2	1	2	1	2	1	2	1
Total		**200**	**100**	**200**	**100**	**200**	**100**	**200**	**100**	**200**	**100**	**200**	**100**

Table 12: Physical characterizations of Bi-layer tablets.

BATCH CODE	AVG. WT. (mg)	HARDNESS(kg /cm2)	Thickness (mm)	DRUG CONTENT (%)		FRIABILITY (%)
				Nebivolol	Valsartan	
B1	304.3±1.24	17.3±4.31	3.91±2.31	91.27	95.65	0.58±2.36
B2	296.0±2.72	15.93±5.21	3.89±2.35	90.75	95.49	0.56±4.21
B3	306.2±4.24	17.34±6.32	3.91±3.52	91.27	95.65	0.54±1.23
B4	303.2±3.21	18.67±3.5	3.92±4.12	91.27	95.65	0.58±4.21
B5	302.9±1.52	17.77±2.31	3.91±4.36	90.75	95.65	0.55±2.36
B6	304.2±3.23	12.64±4.21	4.13±2.42	91.27	95.49	0.56±4.25

All values are mean ± SD, n=3,

In vitro dissolution study

All parameters, conditions and procedure for the study was similar to the phase I dissolution studies.

Table 13: Drug Release from Valsartan sustained release (SR) layer

	B1	B2	B3	B4	B5	B6
Time Hrs	%CDR	%CDR	%CDR	%CDR	%CDR	%CDR
0	0	0	0	0	0	0
1	11±8.94	12±7.45	18±22.41	11±0.00	9±9.94	10±0.00
4	25±11.50	25±6.20	30±1.36	27±2.03	24±16.81	24±6.46
8	37±7.77	39±8.70	46±3.00	43±4.01	38±14.49	40±2.58
20	64±5.17	66±5.91	80±5.92	79±8.50	70±13.66	72±0.57
24	71±3.64	76±5.92	84±6.76	87±9.33	80±13.80	81±2.92

All values are mean ± SD, n=6. , % CDR is percent cumulative drug release

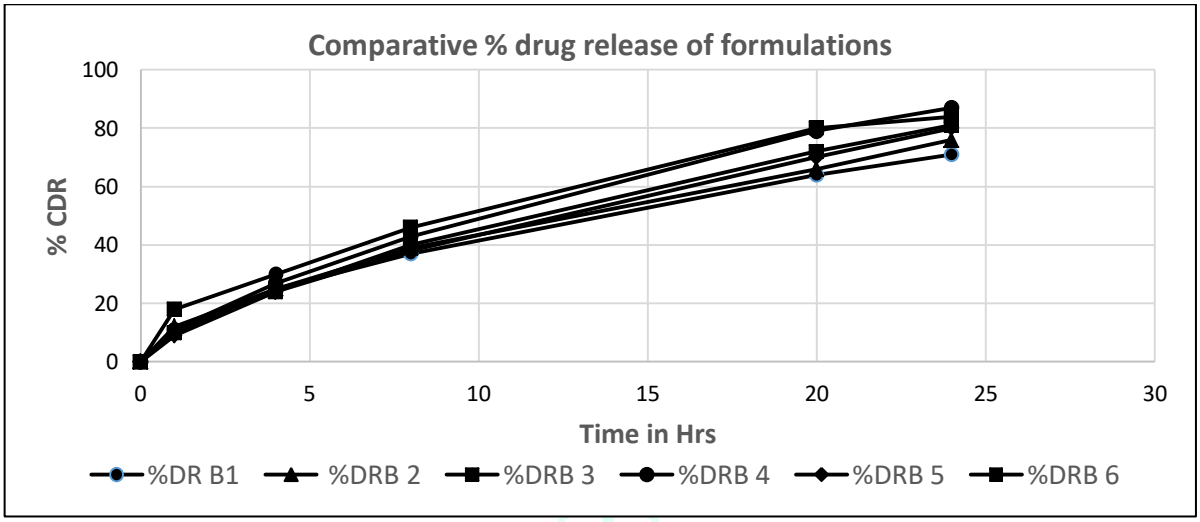

Figure 3: Drug Release from Valsartan SR layer for formulations B1 to B6, %CDR is percent cumulative drug release

Table 14: Drug Release from Nebivolol hydrochloride immediate release (SR) layer

I set Dissolution of IR1 with SR1,SR,SR3	Time mins	
	15	30
B1	65±3.94	79±7.36
B2	71±4.91	83±1.77
B3*	79±6.72	92±3.47
II set DIssolution of IR2with SR4,SR5,SR6		
B4	71±5.49	83±6.17
B5	67±1.12	835.25
B6	74±22.31	81±4.73

All values are mean ± RSD, n=6.

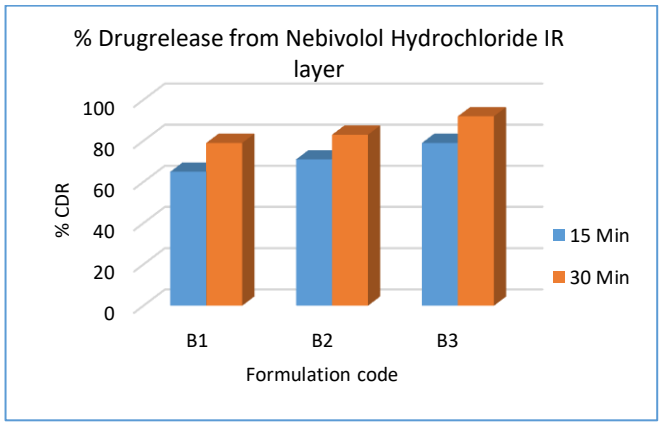

Figure 4: Drug Release from Nebivolol Hydrochloride IR layer for formulations B1, B2 and B3

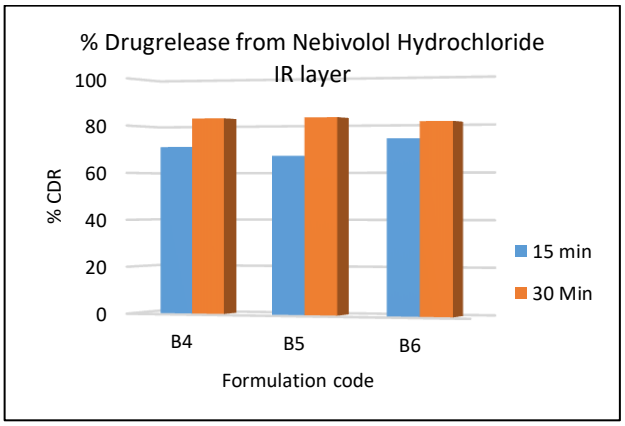

Figure 4A: Drug Release from Nebivolol Hydrochloride IR layer for formulations B4 to B6

The results of immediate release and sustained release layers were compared to analyze the similarities in the behavior.

Table 15: comparative release profile of Amlodipine besylate and Nebivolol hydrochloride from immediate release (IR) layers

Time in min	Formulation code	Amlodipine besylate(%CDR)	Nebivolol hydrochloride(%CDR)
30	F1 vs B1	100±5.08	79±7.36
30	F2 vs B2	108.2±4.6	83±1.77
30	F3 vs B3	103.2±3.53	92±3.47
30	F4 vs B4	107.4±1.48	83±6.17
30	F5 vs B5	105.5±1.5	83±5.25
30	F10 vs B6	96± 0.6	81±4.73
Correlation coefficient	r=0.9894378		

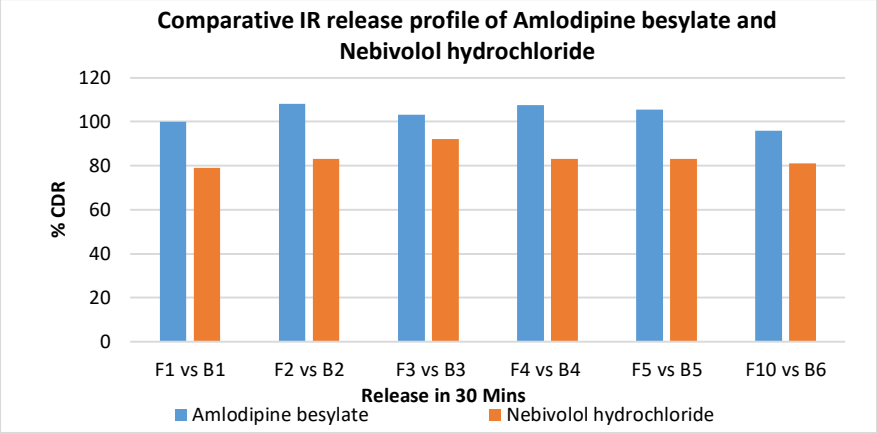

Figure 5: comparative release profile of Amlodipine besylate and Nebivolol hydrochloride from immediate release (IR) layers

Table 16: Comparative release profile of Metoprolol succinate and Valsartan from sustained release layer (SR)

Time hrs.	F1	B1	F2	B2	F3	B3	F4	B4	F5	B5	F10	B6
0	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1	17.0 +3.70	11±8.94	13.0 +4.95	12±7.45	12.6 ±4.83	18±22.41	17.1 ±4.10	11±0.00	21.4 ±5.53	9±9.94	14.3 ±4.66	10±0.00
4	31.0 ±2.53	25±11.50	24.4 ±4.56	25±6.20	25.2 ±3.79	30±1.36	29.5 ±2.96	27±2.03	31.8 ±2.40	24±16.81	33.8 ±4.35	24±6.46
8	51.2 ±2.25	37±7.77	47.1 ±2.84	39±8.70	49.6 ±3.73	46±3.00	47.2 ±3.43	43±4.01	49.7 ±2.81	38±14.49	51.9 ±3.52	40±2.58
20	98.4 ±1.83	64±5.17	99.7 ±1.67	66±5.91	99.5 ±1.42	80±5.92	100 ±1.63	79±8.50	96.1 ±0.70	70±13.66	96.5 ±3.28	72±0.57
Correlation coefficient	0.999925		0.986814		0.990130		0.99445		0.99322		0.999498	

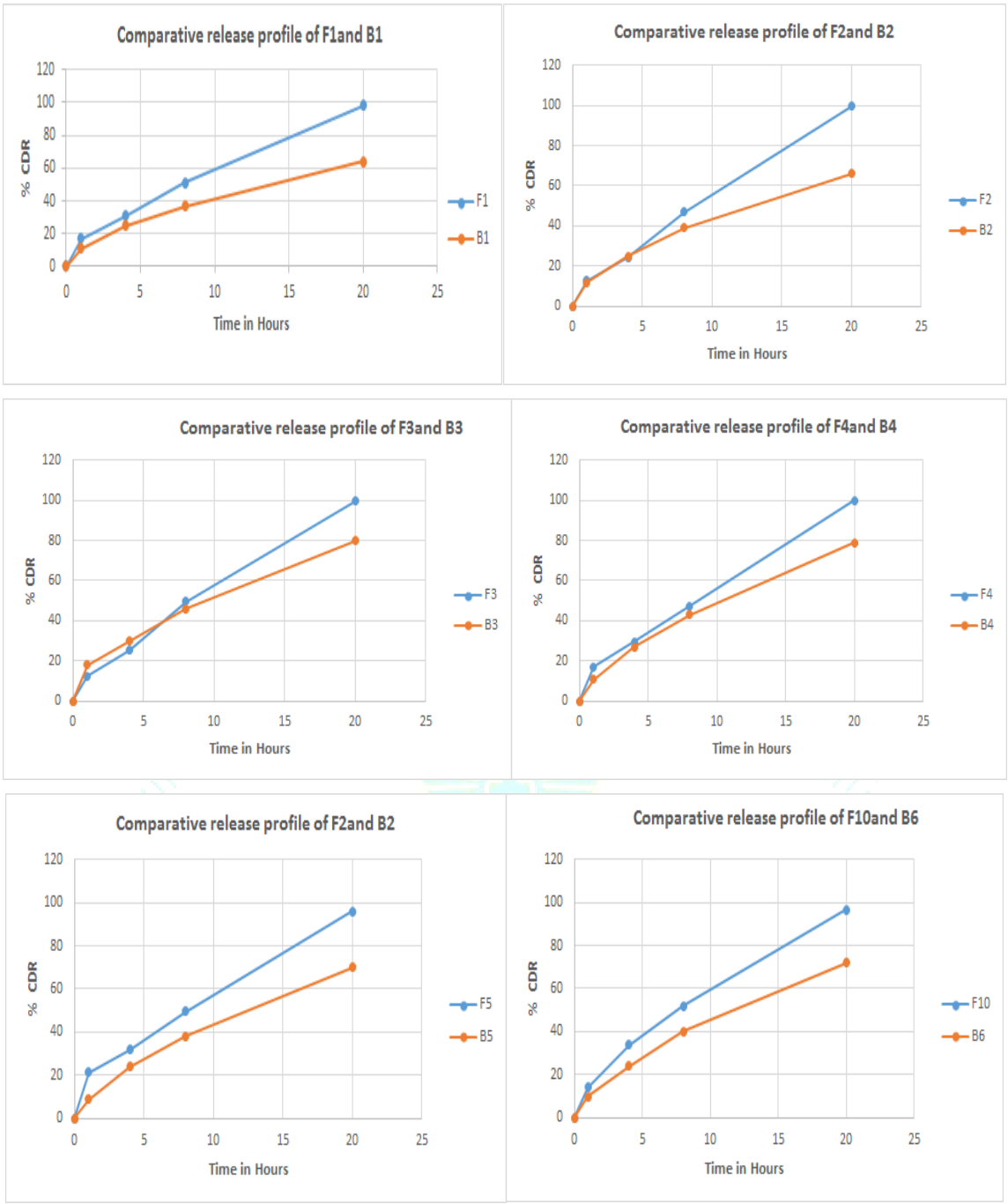

Figure 6: Comparative sustained release profile of model and test drugs (F series contain Metoprolol succinate and B series contain Valsartan)

RESULTS AND DISCUSSION

Different formulations of Bi-layer tablets were prepared and evaluated with an idea to develop Metoprolol Succinate and Amlodipine Besylate as model drugs to be incorporated in sustained release layer and as an immediate release layer respectively. This idea will help in improving hypertension therapy of two drugs and patient's compliance. These two drugs are extensively researched in various formulations for the past two decades. Hence they were considered as model drugs for the present study.

Direct compression method was selected for the formulation. The polymers and other excipients were selected based on the literature survey and satisfying results produced during drug-excipients compatibility studies to develop the final formulation (Table.1). In Phase I experiments, bulk density in the range of 0.3 -0.6 gm/cm^{3}indicates a good packing characteristics. The Carr's compressibility index was found to be in the range of 37-38 % which suggested optimal compressibility. The values of Hausner ratio where found in the range of 1.5 to 1.6 suggested optimal flowability of powder blend. The angle of repose of all the blend was within range of 27 to 28 indicated excellent flow property of powder blend. The bilayer tablets were evaluated for different physical parameter (Table 1). The hardness of bilayer tablet was found in the range of 10 to 12 kg/cm2 which was more as compared to individual layer because of double compression. The thickness of the bilayer tablet was in the range of 3.5 – 3.7 mm which is an excellent value for double layer. The friability was 0.49- 0.54% for bilayer tablet which was less than 1 indicating good handling of tablet. The weight variation study showed low standard deviation uniformity in weight of the tablets 300 ± 0.06mg. (Table 3)

In the composition of Amlodipine Besylate immediate release layer, superdisintegrants sodium starch glycolate and

Croscarmellose sodium were used in the range of 2 to 4 mg per tablet. The results of drug release from IR layer, infers that at the concentration of 2 to 4 mg both the super disintegrants comply with the limit for drug release in 30 min(Table:5, fig 1)

In the formula for Metoprolol Succinate sustained release layer, Hydroxy Propyl Methyl Cellulose (HPMC K 100 M) and HPMC K4M were used as retardant polymers. From the drug release profile it infers that Hydroxy Propyl Methyl Cellulose (HPMC K 100 M) at a concentration of 50 to 60% produced desired release profile for Metoprolol Succinate extended release layer as per USP limits. F1, F2, F3, F4, F5and F10 were considered best formulations. (Table: 6, Fig 2&2A)

To analyze the pattern of drug release, the drug release data of the best formulations were subjected to release kinetics studies. The results show that formulations depict zero order release pattern where the prime mechanism is diffusion controlled (Table. 7).

For phase II experiment, new combination of antihypertensive drugs, Nebivolol hydrochloride and valsartan were chosen based on the similarities with the model drugs Amlodipine besylate and Metoprolol succinate. In the chosen best formulation F1, F2, F3, F4, F5 and F10 keeping all excipients and their composition same, the model drugs were replaced with the test drugs under consideration. Bilayer tablets were formulated in the similar way and all precompression and post compression parameters analyzed in the same manner. The results are shown in (Table: 9, 10, and 12). The results are all within limits and nearly similar to the model drugs. The drug release profile of Nebivolol Hydrochloride is shown in (Table no: 14, fig 4 &4A) and release profile of Valsartan is shown in (Table no: 13, fig 3&3A).

Table 15 shows the comparative release profile of Amlodipine besylate and Nebivolol hydrochloride from immediate release (IR) layers. The r value 0.98943 indicates a good correlation between the release profile of Amlodipine besylate and Nebivolol hydrochloride from the IR layer. The bar graph (Figure 5) of the release of these two drugs in 30 minutes indicate that Amlodipine besylate has greater percentage release (96-107%) at 30 minutes than Nebivolol hydrochloride. This may be due to its high solubility profile. In case of Nebivolol extent of release (79 – 92%) indicates an improvised solubility since it is a BCS class II drugs. This may be due the superdisintegrants sodium starch glycolate and cross carmellose sodium, which enhances the solubility.

Table 16 shows comparative release profile of Metoprolol succinate and Valsartan from sustained release layer (SR). The r value in the range of 0.9998 indicates a good correlation between the release profile of Metoprolol succinate and Valsartan. Figure 6 shows the pattern of release of Valsartan which is almost similar to that of Metoprolol succinate in all formulations. The percentage drug release of Valsartan is less at the consecutive time points when compared to Metoprolol succinate. This might be due to its low solubility profile.

CONCLUSION

The present research was planned to develop an ideal dosage form that shall provide an optimal therapy for hypertension satisfying the patient requirement at the same time be formulation friendly. The ideology was to first standardize the process variables with well-accepted drugs so that the incorporation of a new combination drug would provide predictable results with a minimal trial runs. The results predict that if two drugs are similar in their physicochemical and pharmacokinetic parameters, their behavior will be comparable provided formulation variables remains constant. This study is a preliminary approach to develop a drug delivery system that could pave way to successful development of novel drug delivery system with the available generic drugs as model drugs. This approach can minimize the time and effort taken in developing a drug delivery system for new drug combinations. This work needs further extensive studies to justify this ideology.

REFERENCES

1. Mahmud A, Feely J. Low-dose quadruple antihypertensive combination: More efficacious than individual agents: A preliminary report. Hypertension. 2007; 49:272–5.
2. Ezzati M, Lopez AD, Rodgers A, Vander Hoorn S, Murray CJ Comparative risk assessment collaborating group. Selected major risk factors and global and regional burden of disease. Lancet. 2002; 360:1347–60.
3. https://www.colorcon.com/products-formulation/all-products/download/677/.
4. Martin A. Swarbrick, J and Cammarata. A., Physical Pharmacy, 2nd Edition, 1983: P.532-533, 502, 513-515.
5. Boris Y. Shekunov,1,4 Pratibhash Chattopadhyay,1 Henry H. Y. Tong,2 and Albert H. L. Chow3; Particle Size Analysis in Pharmaceutics: Principles, Methods and Applications, Pharmaceutical Research, Vol. 24, No. 2, February 2007 (# 2006)
6. A Complete Solution for Method Linearity in HPLC and UHPLC. Frank Steiner, Fraser McLeod, Tobias Fehrenbach, and Andreas Brunner Dionex Corporation, Germering, Germany
7. A Complete Solution for Method Linearity in HPLC and UHPLC. Frank Steiner, Fraser McLeod, Tobias Fehrenbach, and Andreas Brunner Dionex Corporation, Germering, Germany
8. A Complete Solution for Method Linearity in HPLC and UHPLC. Frank Steiner, Fraser McLeod, Tobias Fehrenbach, and Andreas Brunner Dionex Corporation, Germering, Germany
9. Sander, Gary & D Giles, Thomas. (2015). Nebivolol and valsartan as a fixed-dose combination for the treatment of hypertension. Expert opinion on pharmacotherapy. 16. 763-70. 10.1517/14656566.2015.1020790.
10. Thomas D. Giles, John R. Cockcroft, Bertram Pitt, Abhijeet Jakate, and Harold M. Wright: Rationale for Nebivolol/valsartan combination for hypertension: review of preclinical and inical data. Journal of Hypertension, Volume 35 _ Number 9 _ September 2017
11. Mazza, Alberto & Gil-Extremera, Blas & Maldonato, A & Toutouzas, T & Pessina, AC. (2002). Nebivolol vs Amlodipine as First-line Treatment of Essential Arterial Hypertension in the Elderly. Blood pressure. 11. 182-8. 10.1080/080370502760050421.
12. Formulation and evaluation of valsartan sr tablets using hydrophilic and hydrophobic polymer L. P. Hingmire* , D. M. Sakarkar Dept. of Pharmacy, Jagdish Prasad Jhabarmal Tibrewala University, Chudela, Dist.Jhunjhunu, Rajasthan, India *Corresponding author: E-mail: lax_hingmire@rediffmail.com, phone no. +91-9422169470
13. Burges R, Moisey D. Unique pharmacologic properties of amlodipine. Am J Cardiol 73 (3): 2A9A, 1994.
14. Van Zwieten PA. Amlodipine: an overview of its pharmacodynamic and pharmacokinetic properties. Clin Cardiol 17 (9 Suppl 3): III3-6, 1994
15. Beta-blockers: their properties and use in hypertension: Prescriber, 5 September 2010. www.prescriber.co.uk
16. Isha shah, shailendra bhatt, alpesh yadav: Enhancement of solubility and dissolution of nebivolol by solid dispersion technique Int J Pharm Pharm Sci, Vol 6, Issue 7, 2014,566-571
17. Manpreet kaur: Enhancement of dissolution properties of amlodipine besylate using liquisolid technique: Int J Pharm Pharm Sci, Vol 5, Suppl 3,2013, 394-400
18. Deshpande, Praful. (2015). Role of components in the formation of self-microemulsifying drug delivery systems. Indian Journal of Pharmaceutical Sciences. 77. 249-257.
19. A. K. Gurram, p. B. Deshpande, s. S. Kar1, usha y. Nayak, n. Udupa and m. S. Reddy* Role of Components in the Formation of Self-microemulsifying Drug Delivery Systems, Indian J Pharm Sci 2015;77(3):249-257.
20. Martin A. Swarbrick, J and Cammarata. A., Physical Pharmacy, 2nd Edition, 1983: P.532-533, 502, 513-515.