Atenolol And Nifedipine Combination Is Better Than Monotherapy: A New Era In Novel Drug Delivery For Hypertension

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Abstract

Hypertension is considered a major health problems globally affect millions of patient.Various study confirms that single drug treatment usually is not adequate to achieve blood pressure goal in most hypertensive patients.In this regard,consideration is given to combination therapy, which offers the potential advantages towards minimizing hypertension in a rapid manner and produces lower adverse effects.SLTs (Sublingual tablets) provides immediate action to enhanced absorption and bioavailability rate. Sublingual tablets are absorbed within the mucus membrane and directly reach in blood systemic circulation. The objective of this ongoing research focused on theatenolol (ATN)and nifedipine (NIF) combined drug deliveryin emergency condition of hypertension. Direct compression technique is used to formulate SLTsby taking different types and concentrations of superdisintegrantsCroscarmellose sodium (CCS) and Crospovidone (CP). Sublimating agents like camphor (CM) and thymol (TY)) also added for better result. FTIR and DSC analysis confirms the ccompatibility results between drug and superdisintegrants.Formulated tablets are evaluated for different parameters and found satisfactory. Formulation F6 considered as the best formulation. The disintegration shows 13 sec and dissolution profile shows 97.36% drug release at 10 min. Formulation F6 shows better pharmacokinetic activity and antihypertensive activity in compare to pure drug and marketed formulation.A combination of ATN and NIF produces rapid disintegration and dissolution property during an emergency and is a lifesaving approach in hypertension treatment.

Keywords: Combination therapy; Hypertension; Direct compression; Atenolol; Nifedipine

Introduction

A few investigations were directed by the specialists in a preliminary premise to affirm that individual medication couldn't be ready to control hypertension in several patients [1]. The sensitivity to an anti-hypertensive drug varies among hypertensivepatients. Because of the synergistic impact of blend treatment minimum quantity of active drugs is useful towards the decrease of hypertension [2]. For example, young people having high plasmarenin activity are sensitive to ß-blockers, angiotensin-convertingenzyme inhibitors or angiotensin II type 1 receptor blockers. In “elderly or those with low plasma renin activity are sensitive to calcium channel blockers” [3, 4]. It is clear that calcium channel blockers can be added to patients with high plasmarenin activity and treated with β-blockers. Similarly, it is not clearthat the addition of β–blockers with calcium channel blockers is better than monotherapy. Here a hypothesis has been made that a combination of β-blockers with calcium channel blockers would be a better choice as an anti-

hypertensive agent and also control organ damage. ATN and NIF, the representative drugs for β-blockersand calcium channel blockers respectively, were used as antihypertensivedrugs in this study.

Atenolol is a ß-blockers that helps in blocking the action of natural chemicals in our body like epinephrine which lowers the heartbeat and blood pressure etc. ß-blockers are the drugs, blockingthe adrenergic receptors enhance the cardiac function such as control heart rhythm, diagnosis of angina and lowdown the blood pressure. β-blockers are also useful for the patient from a second heart attack and control the heart rate by relaxing the blood vessel for easy blood flow.

Nifedipine as calcium antagonist produce a synergistic effect in a combined form with a high potency in angina treatment. During the combination therapy of nifedipine and atenolol, oxygen demand can be reduced in the primary stage of myocardial infarction whereas the vasodilator effect of nifedipine,enhance the supply of oxygen. Monotherapy of ATN and NIF provide prophylactic treatment but in some situation combination form is more preferable.

Several innovations have been made in drug delivery but oral drug delivery still considered the most preferable route for drug administration. Oral drug delivery provides better therapeutic effect due to accurate dosing, cost-effective therapy, self-medication enhance patient compliance. Sublingual tablets (SLTs) are disintegrate in saliva in a fraction of time in the absence of water [5]. Numerous methods have been developed to formulate SLTs such as wet granulation, spray drying, freeze-drying, direct compression, molding, and sublimation method, etc. [6-8]. The advantages of the SLTs are faster dissolution properties with improved bioavailability, high surface area, and efficient production. Superdisintegrants significantly used in the formulation of SLTs [9-11]

The current research focused on the formulation and development of combination therapy between ATN and NIF SLTs considering superdisintegrants at different ratio by sublimation technique. Developed SLTs evaluated by different parameters, in a comparison with pure drug and marketed formulation.

Materials and methods

Nifedipine powder was a gift sample fromEmcure Pharma, Pune, India. Atenolol procured from Sigma Aldrich, India.Crospovidone was procured from the Nice laboratory, India.Croscarmellose sodium is obtained from Signet, Mumbai. Similarly, camphor, aspartame, microcrystalline cellulose, magnesium stearate, and talcwere of analytical research-grade and used as received from Divya Chemicals, India.

Formulation of ATN and NIF loaded SLTs

The sublimation method has been selected to formulate combination therapy of SLTs of ATN and NIF with Superdisintegrants (CCS and CP) at different ratios. Combination therapy considered to formulate seven formulations differently in a manner that formulation F1 as control doesn't have any sublimating agent with an equal ratio of CS and CP. Formulation F2 and F3 containing 10% camphor with 20% CP and 10% thymol with 20% CS respectively. Formulation F4 and F5 contain an equal amount of CP and CS with 15% camphor and thymol as sublimating agents respectively. Similarly, a variation between disintegrating and sublimating agents such as CP (5% and 15%), CS (15% and 5%), camphor (10% and 5%), and thymol (5% and 10%) observe in formulation F6 and F7 respectively. All ingredients have passed through a #80 mesh screen before mixing. The screened substances were mixed intimately without magnesium stearate.For lubrication magnesium stearate is added to the mixture shown in Table 1.The powder mixture of all formulations are evaluated for pre-formulation studies (Bulk density, tapped density, Carr's index, Hausener's ratio and angle of repose). Evaluated parameter for all formulation represent in Table 2. The powder blend was slugged and compressed to obtain a tablet by using direct compression technique. "For sublimation of camphor and thymol SLTs are kept inside hot air oven at 60^{o}C for 8 h to obtained a poroussurface" [12].

Table 1: Formulation of ATN and NIF loaded SLTs (mg)

Ingredients	Batches						
	F1	F2	F3	F4	F5	F6	F7
Nifedipine	10	10	10	10	10	10	10
Atenolol	25	25	25	25	25	25	25
Crospovidone	15	-	20	10	10	5	15
Croscarmellose sodium	15	20	-	10	10	15	5
Camphor	-	10	-	15	-	10	5
Thymol	-	-	10		15	5	10
Aspartame	5	5	5	5	5	5	5
Lactose	25	25	25	20	20	20	20
Magnesium stearate	5	5	5	5	5	5	5
Net weight	100	100	100	100	100	100	100

Drug compatibility study

Analytical technique such as FTIR and DSC were used to determine the compatibility of active drug with excipients.In this study, drug and excipients interaction can be determined based on functional group displacement of the compounds confirms the compatibility [13]. The analysis of spectrum have been carried out for pure drug, excipients, and its mixtures to find out any deviation in functional groups (shifting of the band or broadening in spectra) [14].In this current research article, ATN and NIF interaction with superdisintegrants carried out by FTIR and DSC studies.

Fourier transform infrared spectral analysis (FTIR)

The samples (ATN, NIF, superdisintegrants and its mixtures) have been analyzed in the wave range between 4000-400 cm^{-1} for FTIR (Shimadzu, Japan) studies to confirm the compatibility study[15,16]. By adapting a KBr-pressed disk technique, KBr is added with the sample (pure drug, excipients, and mixtures) in a 10:1 ratio respectively and finally, a semitransparent pallet has been formed and submitted to the FTIR analysis. Potassium bromide (KBr) is the commonest alkali halide taken for pellet preparation because of high transparency which helps the light to be transmitted to the detector [17-19]. FT-IR analysis used to identify unknown materials and for the determination of purity andcompatibility of the compound.

DSC study

Differential Scanning Colorimetry (DSC)analysis a graph between molecular weight and temperature (10^{o}/min from 0 to 700^{o} under nitrogen flow rate 50ml/min). the resultant spectrum shows exothermic or endothermic reactions for the sample which depends on the analysis techniqueadapted. DSC takes the first preference for rapid analysis of incompatibility of the pharmaceutical mixture Thus, in this current research DSC (SDT.Q600, USA) is a thermoanalytical method used to study interaction between active drug and excipients. The pure drug,superdisintegrants, and its mixtures were scanned individually to confirm compatibility [20,21].

Post evaluation study of ATN and NIF loaded SLTs

Weight variability study

Randomly selected twenty SLTs from individual formulation were collected to determine the uniformity in the weight of tablets. Individually

weighed all the tablets using weighing balance (Ohaus, USA). Weight variation for all formulations represent in Table 2

Tablet thickness test

Varnier caliper is used to find out the thickness of the SLTs. Tablets are placed in between the openings of varnier caliper. Randomly selected 3 tablets from each formulation considered to calculate average thickness presented in Table 2[22].This test will provide information about physical dimension of formulated tablets and its uniformity.

Tablet hardness test

Hardness played an important role in transportation. The unit for the hardness is kg/cm^2.The force required to break or crack the tablet noted down by using Monsanto hardness tester. The average is represented in Table 2.

Friabilitytest

Roche friabilator (Veego instrument corporation, Mumbai) is used to conduct tablet friability test. Randomly 20 tablets were selected from each batch and weighed properly (W_0), after friabilator (25 rpm for 4 min) reweighed the tablets (W). Percentage of friability calculated by the equation mentioned below. Standard limit for % friability is below 1% [22].

$$\% \text{ friability} = (W_0 - W)/W_0 \times 100$$

Wetting Time

A simple step has been followed to calculate wetting time of the formulated tablets. A double folded tissue paper was placed inside the petri plate having 6ml distilled water. A water-soluble die (amaranth) added gradually to color the water. Tablets were placed on the tissue paper and the end time was noted, when a completely color (red color) change is observed [23].

Drug content

Ten tablets randomly selected from each groups were powdered by mortar and pestle. The average quantity of powder dissolved in 15 ml methanol in a conical flask. The solution was further sonicated till a homogeneous solution was obtained. Obtained solution filtered (Whatman filter paper) and after suitable dilution absorbance measure with UV Spectrophotometer (UV-1800 Shimadzu) for ATN and NIF at 224 nm and 237 nm respectively [22].

pH of the SLTs

Formulated SLTsinvestigated for pH to avoid irritation.If pH of SLTs deviated more towards highly acidic or alkali could damage the mucus membrane. So the obtained pH of a SLTs should be similar to saliva. Absorption of drug could be enhanced if SLTs pH maintained properly [24]. The digital pH meter used to identify pH of a SLTs.

In-vitro disintegration test

The in-vitro disintegration test is conducted to find out the breaking time of a SLTs using disintegration apparatus (Veego, India). Tablets are inserted inside the tubes present in the basket and moved longitudinally [25]. Distilled water as disintegrating medium taken to conduct the test at 37±0.5^{o}C temperature.

***In vitro* dissolution test for SLTs**

Single basket type dissolution apparatus (Lab India, DS 8000) is used to determine the percentage of drug release from the formulated SLTs[22]. Randomly selected SLTs from each formulation considered for the test. Dissolution results may differ due to different types of superdisintegrats and sublimating agents at various concentrations in each formulation of SLTs. The dissolution apparatus contains a 900 ml buffer solution pH 6.8 at 37±0.5^{o}Cand the shaft rotates at 100 rpm. A sample (5mL) of aliquot was withdrawn at different time interval and replaced the same to maintain the concentration. After a suitable dilution collected samples were analyzed under UV Spectrophotometer to analyze ATN and NIF at 224 nm and 237 nm respectively [22].

***In-vivo* studies**

Animal selection

In this study all the animals (Male Wistar albino rats) are housed under the standard conditions and clinically examined. Animal were provided free access to pellet, food, and water.The animals were preserved in a cleanroom maintaining temperature 20-25^{o}C with a 12 h light and dark cycle and controlled RH 60-70%.

All the animals divided into 4 groups having 6 animals each (age: 4-8weeks; weight: 200-250g). The animal ethical clearance was approved before performing the study.
Group-I contained control animals
Group-II was administered with a pure antihypertensive drug
Group-III formulation F6 (Best formulation)
Group-IV comprised of marketed antihypertensive tablets (Aten 25 mg Tablet (ATN), Calcigard 10 mg Tablet (NIF))

***In-vivo*Pharmacokinetic Studies**
All the animals kept overnight fasting before the experiment and food were provided during the post 2 h administration of the dosing. The animal restraint device is used to expose the head of the animal during SLT administration. Both the jaws of the mouth were separated by the help of wood made tongue depressor to place the SLT easily. 2ml of water was supplied to disintegrate the tablet. To avoid chewing animals mouth was kept open for 1 min using gentle strain to allow complete disintegration of SLTs. Blood samples (0.2ml each) were collected from the retro-orbital plexus/tail vein of the pre-anaesthetized animals at the regular time intervals(0.5,1,1.5,2,3, 4, 6, 8, 10,12, 18 and 24 h) after the administration of the tablet. Blood samples were centrifuged for 10 min at 3000rpm for the extraction of plasma. HPLC technique is used to analyze drug content [26,27]. The obtained data used to determine pharmacokinetic parameters. The obtaineddata were fitted to one compartment open model and analyzed for pharmacokinetic parameters (C_{max}, t_{max}, AUC_{o-t}, Ke, $t_{1/2}$) using PK solver software.

Hypertension induction
Subcutaneous administration of 20 mg/kg/week, dose of methylprednisolone acetate once a week for two weeks to induce hypertension. Methylprednisolone acetate retain salt in the animal model became hypertensive. The blood pressure was frequently monitoredfor the animals and the systolic pressure range in 160-190 mm of Hg was considered as a hypertensive rat [28, 29]

Blood pressure measurement:
All the selected hypertensive animal was segregated into groups. All groups except the control induced hypertension by methylprednisolone acetate. To find out the antihypertensive effect, animal models feed with a subsequent amount of dose.At the prescribed time interval systolic blood pressure was checked.Small animal tail noninvasive blood pressure system based on tail-cuff technique is adopted to check the blood pressure for each animal. The systolic blood pressure recorded by a non-invasive tail-cuff system using the CODA II TM.

Stability study
Stability studies for the best formulation under a controlled environment by maintaining Indian weather conditions is needed to estimate shelf life. The chemical stability can be performed by several evaluation tests for the best formulation. Stability study performed in a stability chamberas per ICH (international conference of harmonization) guideline for six months at $40^{o}C \pm 2^{o}C$ / 75% ± 5% RH. Various stability parameters e.g. physical appearance, drug content,*in vitro* dissolution and *in vitro* disintegration studies were evaluated in triplicate.

Results and Discussion
Properties of mixture
Preformulation is a set of research studies that concentrate on the physicochemical residences of a brand new medicine, would affect the drug's overall formulation of dosage form and its performance. To decide the character and analysis of properties of given compound bulk and tapped density are the critical parameters to be conducted. These residences of the given compound may also vary because of the crystallization and homogeneous blending of the compounds. The density of the sample mixture additionally impacts the compression and flow property of the final product. Preformulation studies have done forATN and NIF combination, for all seven formulations with different excipients at various ratios reported in Table 2. Obtained data confirms that the bulk density of the formulations lies between 0.384 to 0.555 gm/ml, tapped density varies between 0.434 to 0.625 gm/ml, angle of repose range between

25.98 to 31.03, Carr's index between 8.18 to14.28 and Hauser's ratio value foundin between 1.08 to 1.16. Obtained results were as per the limit and observed excellent flow property for each formulations.

Table 2: Pre-formulation study ofATN and NIF loaded powders(Formulation F1- F7)

Pre-Compression Parameter	F1	F2	F3	F4	F5	F6	F7
Bulk density	0.454±0.13	0.416±0.51	0.555±0.41	0.476±0.61	0.384±0.11	0.416±0.81	0.454±0.77
Tapped density	0.515±0.41	0.480±1.73	0.625±0.12	0.555±0.51	0.434±0.92	0.458±0.63	0.495±0.81
Angle of repose	25.98±1.07	28.15±0.75	30.88±1.09	31.03±0.83	29.16±0.03	26.93±0.84	29.01±0.04
Carr's index	11.81±0.52	13.33±0.61	11.11±53	14.28±0.91	11.53±1.05	9.16±0.72	8.18±0.63
Hausner's ratio	1.13±0.71	1.15±0.91	1.12±0.81	1.16±0.75	1.13±0.92	1.10±0.51	1.08±0.48

Compatibility studies

All the drugs showsphysical, chemical, and therapeutical properties that need special consideration before the final formulation. These points need to be focused to decide the ratio of the drug to combine with the appropriate excipients within a specific limit before the final dosage shape. The objective behind the preformulation study is to take a look at the elegant, stable, powerful, and secure dosage formulation with the aid of using setting up kinetic study profile, compatibility with the excipients, and set up the Physico-chemical parameter ofnew drug development.

FTIR study

To perform this study, theactive drugs, superdisintegrants, and its mixtures were selected to determine the compatibility.FTIR spectra of active drugs (ATN and NIF), superdisintegrants(CCS and CP), and their mixtures represent in figure 1. Spectra for atenolol, CCS, and CP have shown different spectra with different peak positions. CCS and CP show broad peaks whereas atenolol and mixture of atenolol with CCS and CP have shown crystalline peaks. The obtained result reveals that individual superdisintegrants and active drug shows different spectra which are different from each other. FTIR spectra shows that major peaks of pure atenolol at 3348.72, 3161.06, 2967.52, 1631.12, 1516.32, 1238.15 $cm^{-1}$are matching (Highlighted in FTIR spectrum by a straight line) with the mixture of atenolol,CCS and CP. Figure 2 represents the spectra of nifedipine, CP, CCS, and their combinations. Pure nifedipine, CP, and CCS have shown different spectrums. Here also the major peaks appear at 3324.44, 2955.75, 1220.49 cm^{-1} for nifedipine shows a clear match (indicated in FTIR spectrum by a straight line) with nifedipine with CP and CCS.

FTIR result with negligible change in IR spectrum confirms that no chemical interaction found betweenactive drugs (ATN and NIF) with superdisintegrants (CCS and CP). So it concludes that the active drug is compatible and maintained its property.

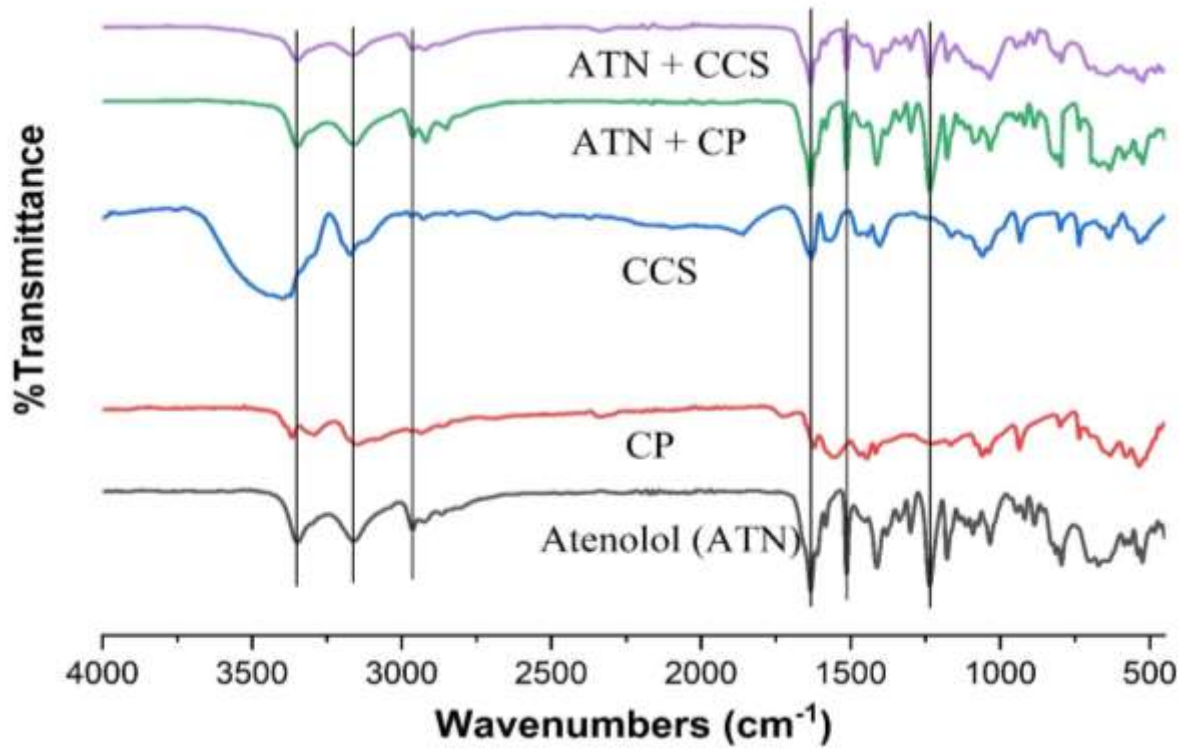

Figure 1: FTIR spectrum for ATN, CP, CCS and its mixtures

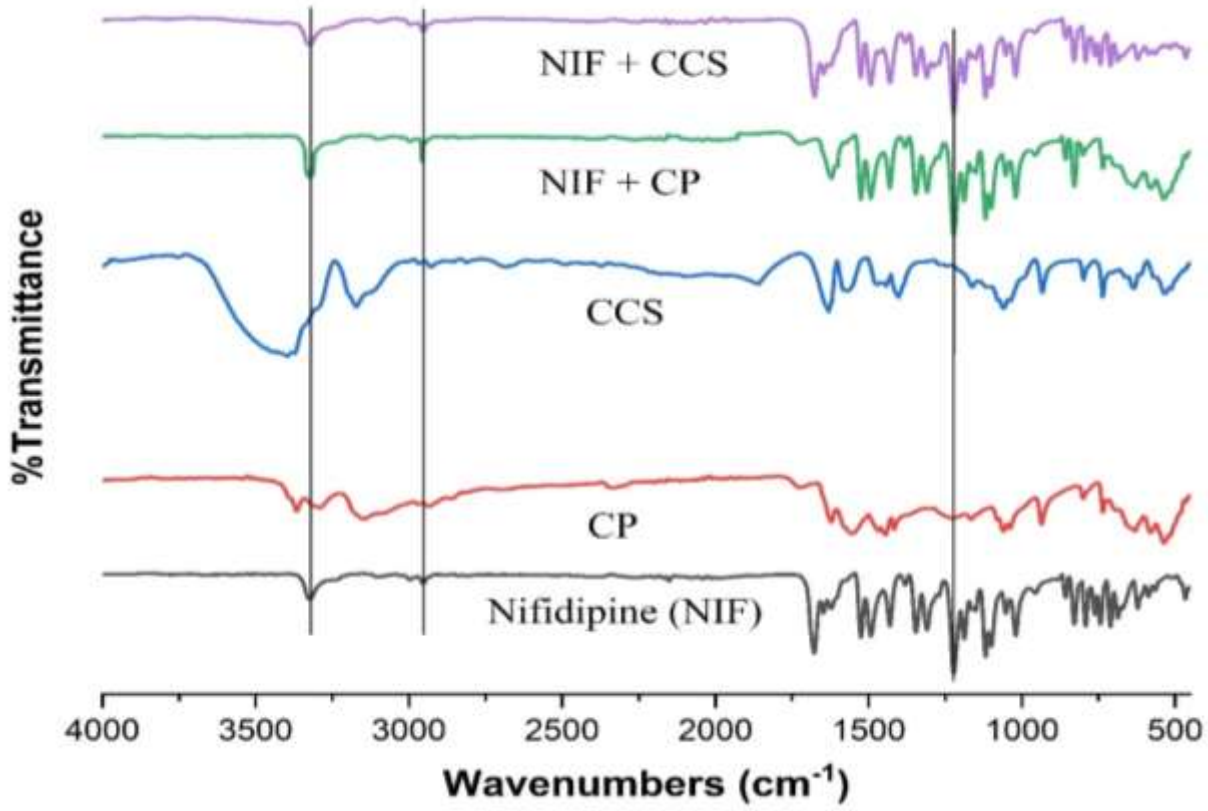

Figure 2: FTIR spectrum of NIF, CP, CCS and its mixtures

DSC study

DSC techniqueis performed to know the compatibility of active drugs (ATN and NIF) with superdisintegrants (CCS and CP)and its mixtures.In DSC observe samples are heated thermally for drug, superdisintegrants, and its mixtures to verify the chemical compatibility. Submitted samples have shown endothermic peaks. Result have shown different endothermic peaksfor atenolol, CP and CCS at 147.79, 130.91, and 154.16^{o}C respectively in figure 3. Whereas atenolol, CP, and CCS mixtures have shown endothermic peaks at 147.79, 149.55, and 150.43^{o}C respectively. Figure 4 reveals that nifedipine, CP, and CCS mixture have shown endothermic peaks at 170.52, 171.57, and 174.12^{o}C respectively. Aminor change ofthe DSC spectrum confirms that the pure drugs (atenolol and nifedipine) have shown good compatibility with superdisintegrants(CP and CCS).

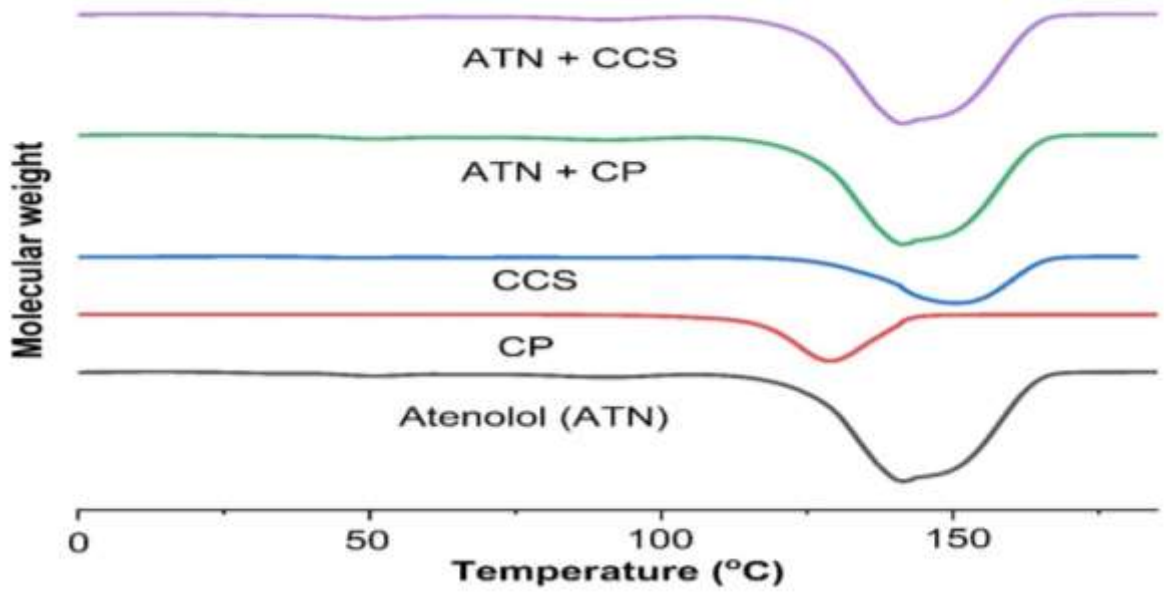

Figure 3:DSC Spectra of pure ATN, CP, CCS and its mixtures

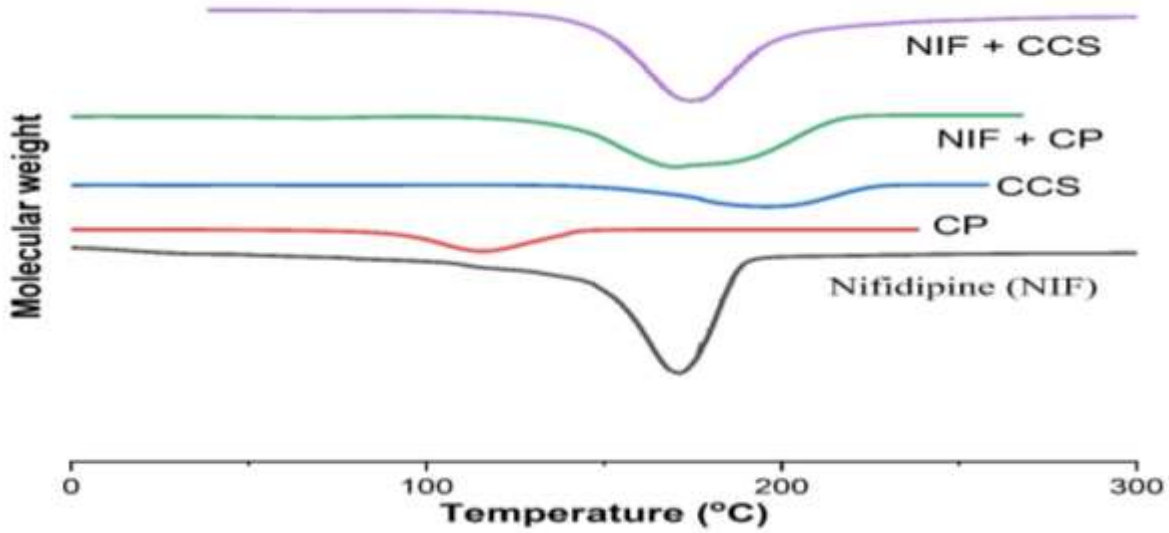

Figure 4:DSC spectrum of NIF, CP, CCS and its mixtures

Physical Evaluation of ATN and NIF loaded SLTs

An evaluation study has been performed for ATN and NIF combined SLTs formulation F1-F7. Table 3 listed all the post-evaluation parameters for all formulations such as average weight of SLTs found to be 99 to 102 mg. Thickness of the SLTs in the range of 2.61 to3.05mm. Hardness for SLTs is 2.32 to 3.03 kg/cm^2. Friability ranges from 0.39 to 0.73% which is below 1%. Similarly, drug content% ranges between 97.8 to 99.75%, surface pH found to be 5.98 to 6.71, andwetting time lies between 20.6 to 30 seconds. In vitro disintegration results shows in the rangebetween 13 to 25 seconds.

Table 3: Post-compression parameters for ATN and NIF loaded SLTs (formulation F1-F7)

Batch no	Tablet Weight variation (mg)	Tablet Thickness (mm)	Tablet Hardness (kg/cm^2)	Tablet Friability (%)	Drug content (%)	Wetting time (sec)	Disintegration time (sec)	Surface pH
F1	102±0.06	2.61±0.02	2.4±0.01	0.41±0.03	99.03±0.06	26.02±0.04	20±0.03	6.5
F2	101±0.41	3.02±0.06	2.32±0.3	0.67±0.04	99.75±0.1	22.1±0.51	15±0.31	5.76
F3	100±0.12	2.63±0.08	2.61±0.06	0.39±0.3	97.81±0.21	23.73±0.8	18.4±0.71	6.37
F4	99±2.32	2.75±0.18	3.01±0.05	0.63±0.02	98.15±1.02	22.63±0.05	16±0.01	5.98
F5	100±0.03	3.05±0.1	2.33±0.16	0.58±0.2	97.8±1.36	23.06±0.01	17±0.2	6.01
F6	100±1.04	2.63±0.51	2.42±0.04	0.73±0.01	99.01±0.06	19.01±0.3	13± 0.7	6.03
F7	100± 0.9	2.73±0.14	3.03±0.1	0.65±0.1	98.74±1.01	20.74±0.04	13.8± 0.03	6.71

Results represent in mean ±SD (n=3)

***In-vitro* dissolution test**

Dissolution study need to perform to know the percentage of drug release of and oral solid dosage form. This test will provide information regarding any changes observed inactive drug or its final product. *In vitro* dissolution results used in R&D for further development of different formulations.

In our current research work, a combination of ATN (calcium channel blockers) and NIF (β-blocker) selected for antihypertensive activity. SLTs are formulated by varying different superdisintegrants (CP and CCS) at different concentrations. Sublimating agents have better diffusion property helps to improved SLTs bioavailability. Combination therapy shows better results than monotherapy. The combination therapy of drugs have been studied earlier by different researchers and observed better synergistic active. Formulation F1 to F7 developed by using combination of ATN and NIF with varying concentration of superdisintegrants and sublimating agents. Obtained results show variation in release raterepresents in figure 5. Formulation F1 contains a combination of ATN and NIF with superdisintegrants CP and CSSat equal ratio (15%) without any sublimating agents shown in Table-1. Percentage of drug release observed in formulation F1 is97.39% at 22 min for NIF and 99.63% at 32 min for ATN. Formulation F2 contains active drugs in a combination with 20% of CCS as and 10% camphor. Formulation F2 contain one superdisintegrants and one sublimating agent. Formulation F2 shows 98% drug release at 16 min for NIF and 98.96% at 22 min for ATN. similarly, Formulation F3 is a combination of active drugs, 20% CP and 10% thymol. Formulation F3, NIF shown 97.82% of drugs released in 20 min but ATN has shown 98.95% at 28. Formulation F2, has shown better drug release than formulation F2 due to the composition of CCS with camphor. CCS has shown two-dimension swelling whereas CP has little swelling property having capillary action

on drug release. This could decrease the drug release in formulation F3. Similarly, camphor is a better sublimating agent than thymol. Therefor CCS with camphor in a combined form enhance in vitro disintegration result of SLTs. In formulation, F4 and F5 contain an equal amount of (10%)CCS and CP but varying with different sublimating agents. Formulation F4 contains 15% camphor whereas Formulation F5 contains 15% thymol. Formulation F4 has shown drug release 98.21% at 12 min for NIF whereas ATN has shown 98.19% at 18 min. Formulation F5 shows 99.71% at 16 min for NIF and 97.99% at 20 min for ATN . Formulation F4 diffuse the drug in a quick manner due to the pores formed by camphor on the tablet surface. Formulation F6 contain CCS and CP at different concentrations like 15% and 5% respectively. In formulation, F6 camphor and thymol used 10% and 5%. In formulation, F6 CCS and camphor concentration are more with respect to CP and thymol to obtain better SLTs. Finally obtaineddrug release percentage for NIF is 97.36% at 10 min and for ATN 99.86% at 16 min in Formulation F6. In formulation F7 we considered the reverse ratio of both sublimating agents and superdisintegrants. Drug release for formulation F7 is 99.81% at 18 min for NIF and for ATN is 99.7% at 24 min. Obtained dissolution result confirms that formulation F6 is the best formulation. So without any superdisintegrants, sublimating agents alone are unable to fast diffuse rate for active drugs. The combined form of both superdisintegrants, sublimating agents helps for fast disintegration. CCS and Camphor composition at higher concentration with CP and thymol consider as better formulation. Superdisintegrantsand sublimating agents combinedly used to formulate better SLTs with improved physiological property helps to disintegrate the tablets quickly. It could provide lifesaving treatment in a hypertensive patient.

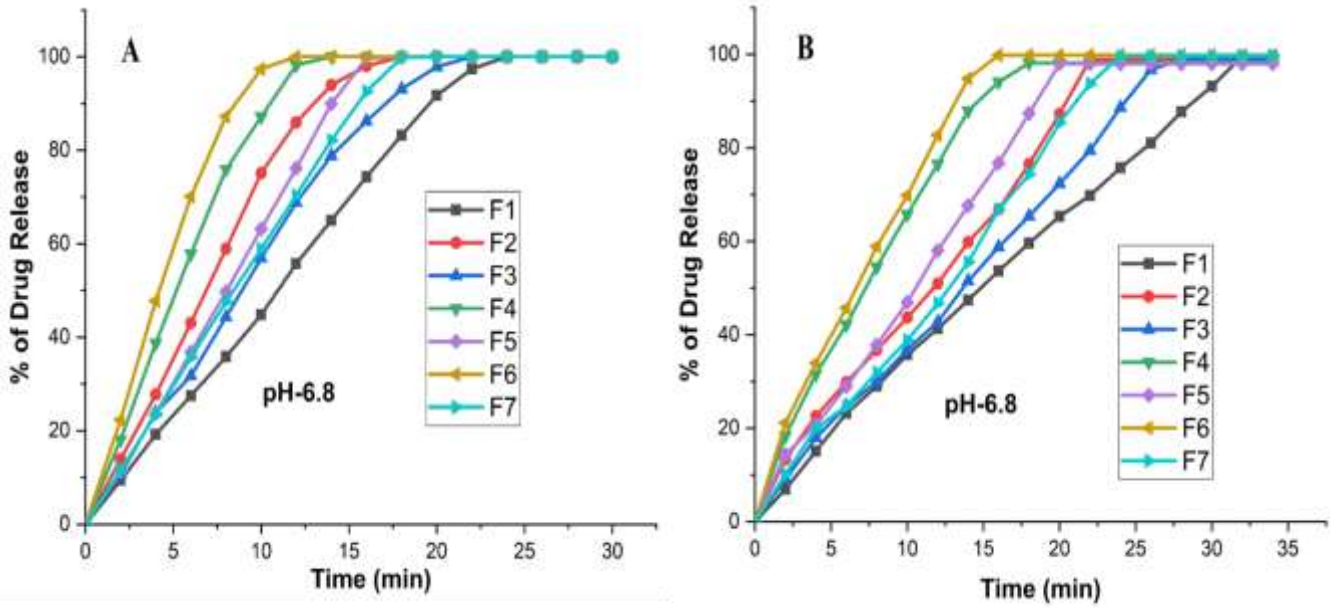

Figure 5:*In vitro* dissolution study of Formulation F1 to F7 (A) NIF loaded SLTs (B) ATN loaded SLTs

Study of *in vivo* pharmacokinetic parameters

For this study healthy Wistar albino rates (male) have been considered and segregate into four groups. Group-I is a control group without induced any hypertensive agent to the rats. Remaining all other groups groups-II, III, and IV, rats are induced hypertensive agents such as methylprednisolone acetate 20mg/kg/week once a week for 2 weeks. Blood pressure measured frequently and the systolic range reaches 180 mm of Hg considered as hypertensive rat. For

group-II pure hypertensive drug (ATN and NIF) was administered. Likewise, Group-III and IV treated with best formulation F6, and marketed antihypertensive drug respectively. Blood samples (0.5 ml) were collected from each group at a regular time intervaland after plasma extraction analyzed under HPLC technique. Various pharmacokinetic parameters (K_E (hr^{-1}), Cl (lit/hr), V_d (lit), C_{max} (µg/ml), $_{max}$ (hr) and $AUC_{(Total)}$ (µg/ml*hr)) were calculated based on plasma data using PK solver software. Obtained results are reported in Table(4and 5) and figure (6 and 7) for ATN and NIF respectively. Pharmacokinetic parameters for ATN in group III and IV have shown better results than group II. Best formulation F6 have better $AUC_{(Total)}$,C_{max}, and Cl compare to marketed formulation and pure drug. Similarly, t_{max} found in formulation F6 is quite less 2.14 h than marketed formulation 2.97. It indicates that in formulation F6, drug release is faster. But in marketed formulation K_E, V_d,have shown better results than the best formulation F6 and pure drug presented in Table 5. Best formulation F6 has shown better in vivo pharmacokinetic result than marketed formulation and pure drug. In Marketed formulation, V_d for NIF is higher than formulation F6 and pure drug. Best formulation F6 has shown better t_{max}1.42 h but both marketed and pure NIF shows similar results 2.33 h and 2.38 h respectively.*In-vivo* pharmacokinetic study reveals that formulation F6 has shown better results and could be useful as anti-hypertensive agent.

Table 4.*In vivo* pharmacokinetic study for ATN

Parameters	Pure drug	Test Formulation (Formulation F6)	Marketed formulation
K_E (hr^{-1})	0.207 ± 0.01	0.094 ± 0.52	0.098 ± 0.05
Cl (lit/hr)	0.683 ± 0.47	1.065 ± 0.16	0.993 ± 0.31
V_d (lit)	5.12 ± 0.61	6.907 ± 0.03	7.47 ± 1.02
C_{max} (µg/ml)	146.03± 5.02	452.88 ± 4.02	290.64± 3.37
t_{max} (hr)	3.48± 0.74	2.14± 0.21	2.97± 0.48
$AUC_{(Total)}$ (µg/ml*hr)	775.6±14.27	1092.49± 22.75	967.59± 13.04

Table 5.*In vivo* pharmacokinetic study of NIF

Parameters	Pure drug	Test Formulation (Formulation F6)	Marketed formulation
K_E (hr^{-1})	0.119 ± 0.21	0.215 ± 0.13	0.113 ± 0.14
Cl (lit/hr)	0.736 ± 0.26	0.847 ± 0.32	0.665 ± 0.26
V_d (lit)	6.14 ± 0.32	3.92 ± 0.63	5.86 ± 0.18
C_{max} (µg/ml)	109.36± 2.14	211.02 ± 3.83	146.64± 3.37
t_{max} (hr)	2.38± 0.35	1.42± 0.11	2.33± 0.21
$AUC_{(Total)}$ (µg/ml*hr)	869.46±11.93	1088.46± 18.15	1196.91± 23.81

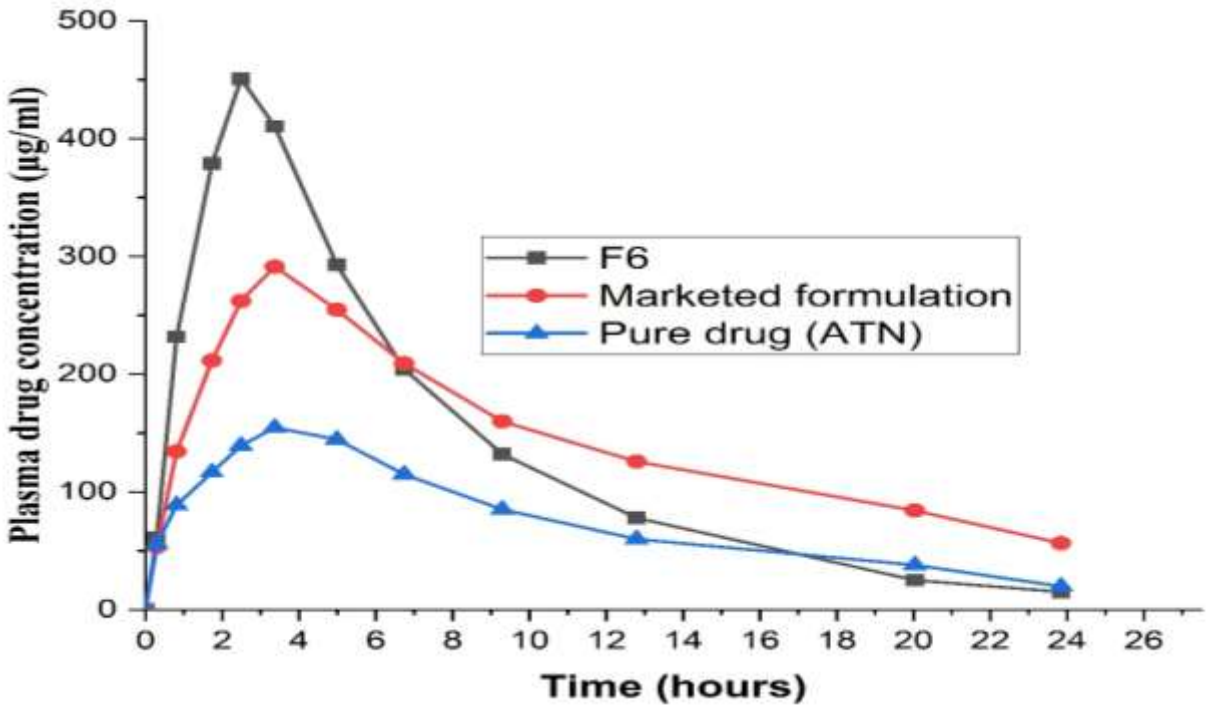

Figure 6: Plasma drug concentration of pure drug (ATN), marketed formulation with best formulation F6.

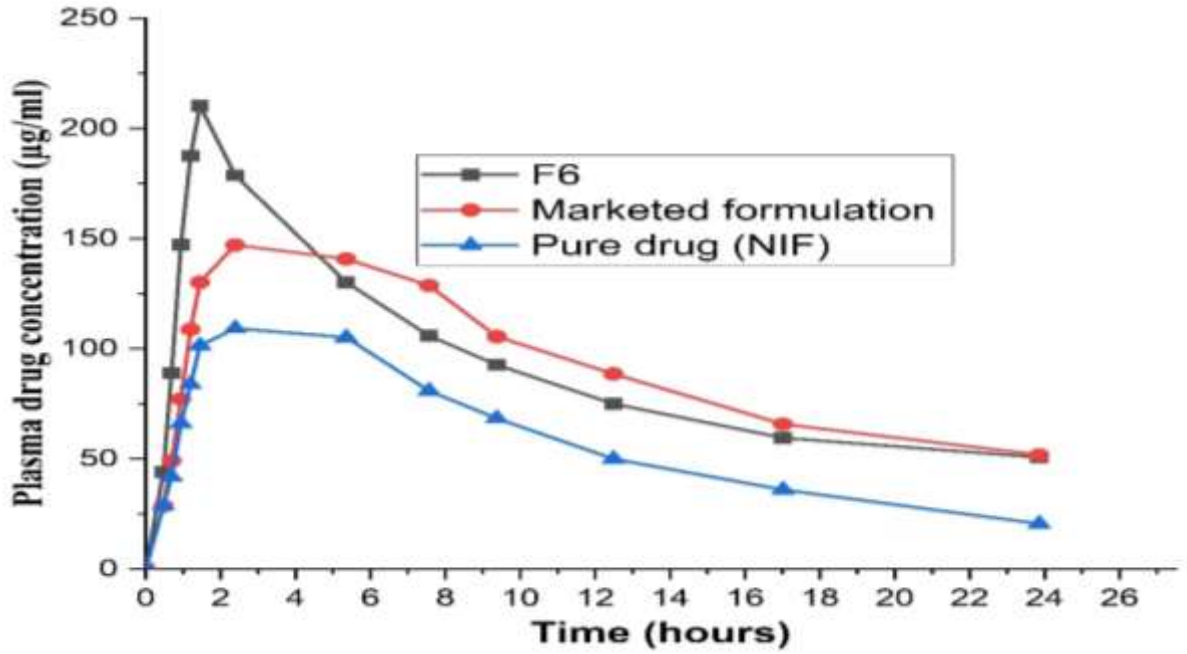

Figure 7: Plasma drug concentration of pure drug (NIF), marketed formulation with best formulation F6.

Antihypertensive activity

The antihypertensive result for all groups represented in figure 8. The antihypertensive activity is carried out when blood pressure (systolic) reaches to 180 mm of Hg in hypertension induced animal groups (Group II,

III and IV). The control group (Group I) rats shows normal blood pressure (systolic) 121 mg of Hg. Obtained results confirm that best formulation F6 produces drastic change in lowering the blood pressure (systolic) from 179.93 to 121.76 in 1 h 30min. But marketed formulation shows reduction of blood pressure 181.01 to 121.73 mm of Hg in 2 h 30 min for NIF and for Marketed ATN has shown 181.35 to 121.63 mm of Hg at 3 h. But pure drug NIF and ATN have shown 180.72 to 121.66 mm of Hg in 3 h 30 min and 180.93 to 121.62 in 5 h respectively. Formulation F6 has better impact on the hypertensive rat model in this study. From this result, it has been concluded that SLTs considered as a best substitute for commercially available hypertensive tablets and pure form of drugs.

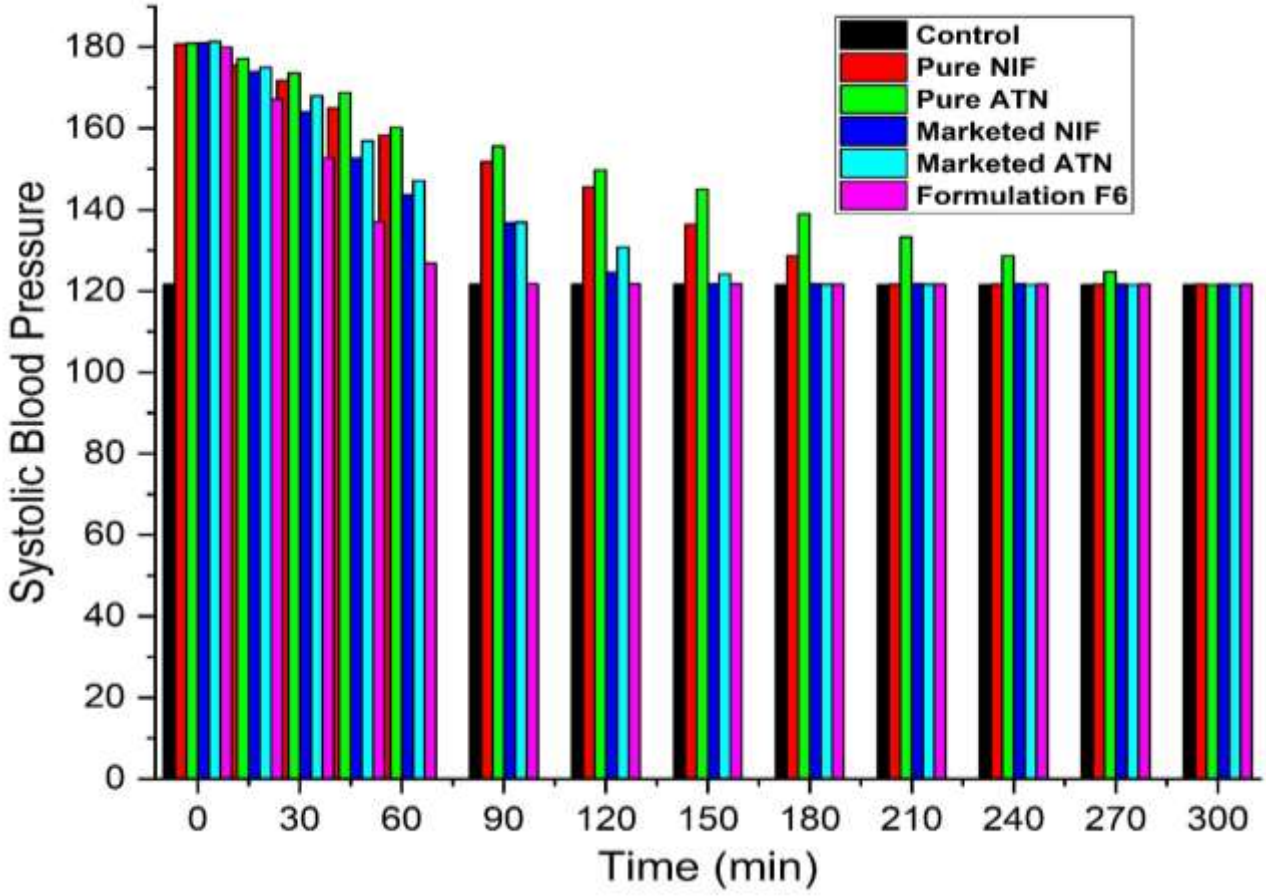

Figure 8:Antihypertensive activity comparison between control, Pure drug (NIF & ATN), marketed (NIF & ATN) and best formulation F6.

Stability study

The selected best formulation F6 is ATN and NIF loaded combined drug delivery for hypertension. Formulation F6 has shown better results in pharmacokinetic studies and antihypertensive activity. Henceforth formulation F6taken for the stability study to determine its self-life shown in Table-6. Self-life is an ultimate goal for the selected formulation. An accelerated stability study has been performed for 6 months and the result reveals that there are no significant changes found in physical appearance (shape, morphology, and color of the SLTs). A slight deviation was observed in results like drug content (0.29%), disintegration time (1 sec), and In vitro dissolution study (1.5%). The overall result confirms that formulation F6is stable.

Table 6: Stability study for formulationF6 SLT

Parameters	Optimized formulation F6			
	0 Day	1st month	3rd month	6th month
Physical appearance drug & excipients	NSC	NSC	NSC	NSC
Drug content %	99.01%	99.32%	98.84%	98.72%
Disintegration time (sec)	13.6 ± .04	13.2 ± .13	14.1 ± .71	14.6 ± .51
In-vitro drug release %	99.71%	99.42%	98.74%	98.21%

All values represent in mean ± standard deviation, (n=3), **NSC:** No Significant Changes

Conclusion

SLTs were formulated in this study in a view of enhancing the treatment facility of hypertension with improved bioavailabilityu.ATN and NIF combinedly loaded SLTs developed aiming to provide a fast disintegration and rapid action of the active dosage form. For a quick disintegration of SLTs a varying concentration of superdisintegrants and sublimating agentsare helpful. It also improves the result of wetting time, in vitro disintegration time and dissolution profile. SLTs quickly disintegrate with saliva without water. FTIR and DSC result confirms the compatibility between drugs and superdisintegrants used in the formulation. Formulation F6 considered as the best formulation based on the results obtained from in vivo pharmacokinetic study and antihypertensive activity. CCS and Camphor composition at higher concentration provide better results for SLTs. Formulation F6 shows 97.36% drug release at 10 min. with better AUC $_{(Total),}$Cmax and Cl results. . Similarly F6 has shown good antihypertensive results in compare to the marketed formulation and pure drug. Formulation F6 combination of ATN and NIF as SLTs hasshown better performance and selected as a useful candidate for hypertension treatment.

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Conflicts of interest:

No conflict of interest.

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