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IN-VIVO ANALYSIS AND OPTIMIZATION OF DUAL-RELEASE MINITABLETS WITH PROLONGED BOSENTAN AND IMMEDIATE SILDENAFIL CITRATE FOR ENHANCED THERAPEUTIC OUTCOME

Deepak Kumar Sarangi[1*], Chandra Sekhar Patro[2], Ch. Niranjan Patra[3] Nalini Kanta Sahoo[4],
[1,2]Centurion University of Technology & Management, Bhubaneswar, Odisha, India
[3]Roland Institute of Pharmaceutical Sciences, Brahmapur, Odisha, India
[4]MIT College of Pharmacy, MIT Campus, ram Ganga Vihar,Phase-II, Moradabad, Uttar Pradesh, India

ABSTRACT

The purpose of this study was to prepare and evaluate multiple unit mini tablets (MTs) of prolonged released(PR)bosentan(BSN) and fast release (FR)sildenafil citrate (SDC) on the basis of encapsulation method for the effective management of pulmonary artery hypertension. The system consists of MTs are in the form of uncoated tablets containing the superdisintegrating agents where as the PRMTs contains the polymer with film coating. All the units are prepared by direct compression method and encapsulated using size 1 hard gelatin capsule shells. The formulations were evaluated fordifferential scanningcalorimetry, dissolution test and post compression studies for their quality attributes and it was found that all the parameters were in the acceptable limits. The optimized formulation for BSN PRMTs i.e BS3 which contains 5% HPMC K15M shows a promising sustained release profile of 82.31% in 24 hours similarly FRMTs of SDC of formulation SD6 that having 3% of magnesium aluminium silicate shows about 90% of drug release within 15 minutes. The in-vivo pharmacokinetic characterization of the system was carried out using wister rats where theAUC value for SDCFRMTs andBSNPRMTswas found to be 94256.88625±123.65ng h/mL and 142438.084±324.11ng h/mL respectively. The other pharmacokinetics are also determined and found to be satisfactory.The six months stability samples showed no significant change in the drug content, hardness and uniformity of the content of the optimized formulations.

Keywords:bosentan, sildenafil citrate, mini tablets, encapsulation, prolong release, fast release, pulmonary artery hypertension.

I. INTRODUCTION

Pulmonary arterial hypertension (PAH) represents a condition seldom encountered, marked by unfavourable alterations in the arterial network, resulting in heightened vascular opposition followed by a rise in right ventricular burden and eventual onset of cardiac insufficiency. Initial manifestations

often lack specificity, commonly manifesting as exertionaldyspnea and fatigue. The present endorsed treatment for PAH encompasses medications augmenting the nitric oxide-cyclic guanosine monophosphate biological pathway which agonists targeting the prostacyclin pathway and antagonists of the endothelin pathway including BSN. Modern therapy consists of a combination of medication regimens that target several biological pathways, including the oxide cyclic guanosine mono phosphate and endothelin and pathways. This strategy has demonstrated observable improvements in health outcomes and mortality when compared to conventional single-pathway targeted medication.

Three crucial mechanistic pathways the prostacyclin, endothelin, and nitric oxide pathways are recognised for their essential role in the advancement of PAH. Therapies focusing on these pathways are readily accessible. Combination therapies addressing multiple pathways offer an enticing approach to PAH treatment, potentially yielding superior long-term outcomes compared to monotherapy. BSN is a groundbreaking accomplishment as the first non-peptide ETA and ETB endothelin receptor antagonist approved for PAH treatment in this particular regimen. With a half-life of 5 hours and an oral bioavailability of 50%, a controlled-release formulation of BSN would be preferable. This approach would help sustain therapeutic plasma concentrations and, consequently, mitigate potential side effects. SDC, a potent and selective orally active phosphodiesterase type 5 (PDE5) inhibitor, is widely distributed throughout the body, with notably high concentrations observed in the lungs. Due to its inhibition of phosphodiesterase type 5 (PDE5), SDC prevents the degradation of cGMP, thereby promoting vascular smooth muscle relaxation and enhancing flow of the blood. This mechanism augments the vasodilatory effects of nitric oxide, leading to improved pulmonary hypertension outcomes.

The objective of the current investigation is to integrate BSN and SDC into a MT encapsulation system for the management of PAH. Utilizing combination therapy would aid in reducing pulmonary hypertension by antagonizing dual receptors and enhancing vasodilation of the pulmonary arteries. Such therapy is beneficial for patients who have not responded adequately to mono therapy. This therapeutic approach not only decreases dosing frequency but also mitigates side effects, thereby improving patient compliance.

II. LITRETURE REVIEW

Naeije et al., 2022, Pulmonary arterial hypertension (PAH) is a rare yet severe condition marked by adverse remodeling of the pulmonary arterial tree, leading to elevated vascular resistance and increased right ventricular afterload, ultimately progressing to heart failure. The disease's nonspecific symptoms and limited awareness of its pathology contribute to delayed diagnosis and treatment, which worsen the prognosis. Recent guidelines emphasize the importance of timely differential diagnosis to distinguish PAH from other types of pulmonary hypertension and advocate treatment tailored to the patient's mortality risk. Advances in diagnostic methods, the development of novel treatments, and the establishment of specialized PAH referral centers have collectively enhanced both the prognosis and quality of life for PAH patients.

Fatima et. al 2018 Pulmonary arterial hypertension (PAH) is a rare and life-threatening disease characterized by adverse remodeling of the pulmonary arterial tree, which increases vascular resistance and right ventricular afterload, ultimately leading to heart failure. Diagnosis is often delayed due to nonspecific symptoms and limited awareness of the disease, resulting in poor prognosis. Current guidelines emphasize early differential diagnosis from other forms of pulmonary hypertension and recommend treatments based on mortality risk assessment. Advances in diagnostic techniques, along with new targeted therapies and the establishment of specialized referral centers, have significantly improved both prognosis and quality of life in patients with PAH. The aim of this study is to assess the impact of early diagnosis and tailored therapeutic approaches on survival and clinical outcomes in PAH patients.

Xu, C., et al. 2022 Gut microbes play a critical role in human health, with orally ingested probiotics effectively enhancing intestinal microbial balance. However, the harsh environment of the digestive tract poses challenges for probiotic viability. Probiotic encapsulation technology offers a promising solution, although traditional methods face limitations, including sensitivity to extreme temperatures and difficulty in achieving optimal microcapsule sizes. Advances in encapsulation technology now range from bulk probiotic encapsulation using nanofibers and nanoparticles to innovative nano “armor” coatings that protect individual probiotics through biofilm and nanocoating techniques. This review explores the materials and systems used in encapsulated probiotic carriers and examines current encapsulation methods for probiotic nanoagents. It further highlights the advantages and limitations of existing systems and discusses the future development and challenges in the field of probiotic encapsulation.

III. RESEARCH METHODOLOGY

BSN and SDC underwent analysis via FT-IR (IR Affinity-1, Shimadzu, Japan) both individually and in conjunction with their respective polymers and also with their respective super disintegrating agent. The MTs prepared underwent various physicochemical tests to assess their pharmaceutical quality, including measurements of weight, thickness, hardness, and friability. Initially, a sample of 20 MTs was randomly selected for weight measurement using an analytical balance (Wensar, High Precision Balance PGB -200). Subsequently, the diameter, thickness, and hardness variations were determined using Pharmatron M50 multi-test instrument. All the MTs are evaluated according to the specified evaluation parameters. The prepared SDC FRMTs and BSN PRMTs underwent an in vitro dissolution study using USP Type 2 equipment. The time intervals for sampling were set differently for SDC FRMTs at 5, 10, 15, 30,45 and 60 minutes and for BSN PRMTs at 1, 3, 6, 9, 12, 15, 18, and 24 hours. The medium of the dissolution was taken as simulated gastric fluid having pH 1.2 (without enzyme). The dissolution jar was refilled with 5 mL of new media after testing samples were removed in 5 mL aliquots to maintain the sink condition. The Institutional Animal Ethical Committee sanctioned the research on assessing SDC and BSN in wister rat blood serum, utilizing UFLC method. For analysis, a system was employed employing a mixture of HPLC grade methanol and phosphate buffer (20mM, pH 4.4) in a ratio of 75:25, which underwent filtration and degassing using a 0.22-µm nylon membrane filter prior to use

IV. RESULT AND DISCUSSIONS

FTIR study confirms the purity of the sildenafil citrate and bosentan. By using the pure drug total 18 numbers of MT formulations has been prepared. The FT-IR for the final formulation of SDC and BSN did not exhibit any major deviation in the absorption bands of pure drugs. Hence it confirms that the drug and excipients are compatible.For the preparation of sildenafil citrate FRMTsdifferent types of superdisintegrants are used. For SD1 to SD3 formulation 1% to 5% of sodium starch glycollate has been used, for SD4 to SD6 and SD7 to SD9 same percentage of magnesium silicate andcrosslinked PVP are used respectively.The use of superdisintegrants in the concentration range of 1 to 5% in the formulation of MTs is justified by several factors that contribute to the effectiveness and efficiency of MTs.Superdisintegrants speed accelerate the breakdown of tablets into smaller pieces, which is necessary for the drugs to dissolve and absorb more quickly. These are cost-effective while retaining their efficacy since they are very effective even at low concentrations. The range offers flexibility in attaining the intended disintegration time and drug release profile by

allowing modifications based on the particular drug and formulation requirements.The concentration range of 1 to 5% for superdisintegrants in MTs is a balance between efficacy, cost, and compatibility, which is crucial for the successful development of MTs that meet therapeutic needs and enhance patient adherence to medication regimens. The rats were administered MTs orally, with each group receiving specific doses of SDC and BSN. Blood samples were collected at predetermined time intervals post-administration to assess drug concentration levels in the serum. Blood samples were collected via the tail vein and processed meticulously to obtain plasma samples for analysis. Proper handling procedures, including centrifugation and storage at appropriate temperatures, were followed to maintain sample integrity[43]. The collected samples were analyzed using validated methods to determine the concentration of SDC and BSN in the serum over time. The obtained data are shows maximum concentration will be 15602±212.12 and 14323±321.29 for both SDC FRMTs and BSN PRMTs respectively. Similarly the value of maximum time of serum concentration are 1.01±0.02 and 4.03±0.03 hours, area under curve values are 94256.88625±123.65ng h/mL and 142438.084±324.11ng h/mL, volume of distribution(V_d) for both the MTs are 17260.51769±23.43 and 83927.75502±12.43 ml respectively. Clearance value for both MTs are 38364.74682 and 24492.72949ml/hr. The mean residence time is 2.12±0.23 and 5.32±0.34 hours for both SDC FRMTs and BSN PRMTs respectively.

Table 1. Formulation of Bosentan PRMTs

Formulation Code	**Quantities (mg/minitablet)**								
	BS1	**BS2**	**BS3**	**BS4**	**BS5**	**BS6**	**BS7**	**BS8**	**BS9**
Bosentan	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Starch	1.7	1.5	1.1	1.7	1.5	1.1	1.7	1.5	1.1
MCC	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
Lactose	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
HPMC K15M	0.2	0.6	1						
Ethyl Cellulose				0.2	0.6	1			
Sodium CMC							0.2	0.6	1
Magnesium stearate	1	1	1	1	1	1	1	1	1
Purified Talc	1	1	1	1	1	1	1	1	1

Total weight (mg)	20	20	20	20	20	20	20	20	20

Table 2: In-vivo pharmacokinetic parameters of prepared MTs

Pharmacokinetic Parameters	**Sildenafil Citrate FRMTs**	**Bosentan PRMTs**
C_{max} (ng/ml)	15602±212.12	14323±321.29
T_{max} (h)	1.01±0.02	4.03±0.03
AUC (ng hr/ml)	94256.88625±123.65	142438.084±324.11
V_d (ml)	17260.51769±23.43	83927.75502±12.43
Clearance CL (ml/hr)	38364.74682±31.33	24492.72949±25.54
MRT (hr)	2.12±0.23	5.32±0.34

Mean±SD, n=5

V. CONCLUSION

In conclusion, this study aimed to develop and assess the effectiveness of multiple unit mini tablets (MTs) containing bosentan and sildenafil citrate for the management of pulmonary artery hypertension.This combination of BSN and SDC as MTs encapsulated into capsules is a novel concept. As this approach is particularly innovative because it combines two different pharmacological actions in one capsule shell, potentially simplifying the treatment regimen and improving patient adherence to therapy. Additionally, the use of MTsin the form of encapsulation is a novel delivery system that may offer advantages over traditional tablet or liquid forms, such as ease of swallowing and the ability to combine different release profiles within a single dosage form.The formulations were prepared using direct compression method and encapsulation with size 1 hard gelatin capsule shells. The MTs were designed to have prolonged release properties for BSN and fast release properties for SDC.The formulations underwent thorough evaluation, including differential scanning calorimetry, dissolution testing, and post compression studies. Results indicated that all parameters met acceptable limits, demonstrating the quality and efficacy of the formulations. The optimized PRMTs formulation for BSN (BS3) showed a promising sustained release profile, while the FRMTs formulation for SDC (SD6) exhibited rapid drug release within 15 minutes.Furthermore, pharmacokinetic characterization conducted in wistar rats revealed favourable results, with significant AUC values for both SDC and BSN

formulations. The stability testing conducted over six months demonstrated no significant changes in drug content, hardness, or uniformity of the optimized formulations.Overall, the findings of this study suggest that the developed microencapsulated MTs have the potential to effectively manage pulmonary artery hypertension. Further research and clinical trials may be warranted to validate these findings and explore their therapeutic benefits in human subjects.

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