FORMULATION AND EVALUATION OF S-(-)-AMLODIPINE BESYLATE AND NEBIVOLOL HYDROCHLORIDE TABLETS

S. A. SHAIKH[*], S. S. SHAIKH[a], S. R. SHAHI[b], M. A. SHOOKUR[c],
L. K. REDDY, A. N. PADALKAR, W. THUBE MAHESH[b]
Jeevanjyoti Pharmaceutical, Aurangabad, M.S, 431210, India.
[a]Kamla Nehru College of Pharmay, Aurangabad, M.S, 431001, India.
[b]Government College of Pharmacy, Aurangabad, M.S, 431005, India.
[c]Mulana Azad College of Arts and Science, Aurangabad, M.S, 431001, India

The objective of the present study was to develop a tablet formulation of S-(-)- amlodipine besylate chiral separation drug and nebivolol hydrochloride for better management of hypertension, while reducing or avoiding undesirable adverse effects, such as headache and edema, dizziness, flushing, palpitation, fatigue, nausea, abdominal pain and somnolence which are often associated with administration of a racemic mixture of amlodipine. The composition containing the optically pure S-(-)- isomer of amlodipine 2.5 mg has calcium channel blocking activity and, nebivolol hydrochloride 5 mg has beta-receptor blocking activity. Combination therapy is essential for control of hypertension. It is well recognized that a single drug, even when used in maximal recommended dosages will control no more than 50% of a hypertensive population. On the other hand, the skillful use of two or more agents in combination can improve hypertension control rates to well above 80%. The present method provides a safe, highly effective method for treating severe hypertension while reducing undesirable adverse effects associated with anti-hypertensive drugs, including the racemic mixture of amlodipine. The study was also carried out to design a suitable dissolution medium for S-(-)- amlodipine besylate and nebivolol hydrochloride. Amlodipine besylate and nebivolol hydrochloride had maximum solubility in pH 1.2 and thus pH 1.2 was selected as the most suitable media for S-(-)- amlodipine besylate and nebivolol hydrochloride dissolution studies. The RSD below 2% indicated insignificant batch-to-batch variation. The accelerated stability study of the optimized formulation was performed as the ICH guidelines. The results indicated no change in optical rotation of S-(-)- amlodipine besylate.

1. Introduction

High blood pressure is estimated to cause 7.1 million deaths, about 13 percent of the global fatality total. It is believed this number will grow to approximately 11 million by the year 2020. Heart disease is the leading cause of death in the U.S., accounting for nearly 740,000 deaths each year. Cardiovascular disease is also the leading cause of death in Europe, accounting for over 4 million deaths each year. Hypertension is the leading risk factor for cardiovascular and renal disease, increasing the risk of myocardial infarction, stroke, congestive heart failure, ruptured aortic aneurysm, and renal disease. It is clearly understandable that a more rational approach to diagnosing and treating high blood pressure could have a substantial impact on population morbidity and mortality, especially considering the current lack of success[1].

---

[*]Correspondence to: shakeel.shaikh11@rediffmail.com

2. Need of combinated therapy

Physicians often have a misguided belief that blood pressure can be controlled with a single drug and demonstrate reluctance to change or to add medications in those patients whose blood pressures are not at recommended goals. Many physicians are inclined to practice sequential monotherapy with individual agents as opposed to recognizing the additive benefits of agents in combination. It is well recognized that a single drug, even when used in maximal recommended dosages will control no more than 50% of a hypertensive population. On the other hand, the skillful use of two or more agents in combination can improve hypertension control rates to well above 80%.

Since no systematic studies on design and development of S-(-)- amlodipine besylate and nebivolol hydrochloride tablet or in vitro are available in literature, we propose to develop a suitable formulation and dissolution medium to characterize in vitro release profile of S-(-)-amlodipine besylate and nebivolol hydrochloride tablet. Thus a safe, highly effective method for treating severe hypertension while reducing undesirable adverse effects with improved patient compliance and acceptance.

The improved dissolution profile and insignificant batch-to-batch variation of the formulated formulation was indicated by RSD below 2%.

Amlodipine is a dihydropyridine calcium antagonist (calcium ion antagonist or slow channel blocker) that inhibits the tran membrane influx of calcium ions into vascular smooth muscle and cardiac muscle. Experimental data suggest that amlodipine binds to both dihydropyridine and nondihydropyridine binding sites. The contractile processes of cardiac muscle and vascular smooth muscle are dependent upon the movement of extra cellular calcium ion influx across cell membranes selectively, with a greater effect on vascular smooth muscle cells than on cardiac muscle cells. The (-) isomer has been reported to be more active than the (+) isomer[2-5].

S (-) amilodipine besilate is a potent drug for the treatment of hypertension while avoiding the concomitant liability of adverse effects associated with the administration of the racemic mixture of amlodipine. The S(-) isomer of amlodipine is also useful for the treatment of angina and such other conditions as may be related to the activity of S (-) amlodipine as a calcium channel antagonist without the concomitant liability of adverse effects associated with the racemic mixture of amlodipine[6,7].

Nebivolol is a racemate of two enantiomers, SRRR-nebivolol (or d-nebivolol) and RSSS-nebivolol (or l-nebivolol). It combines two pharmacological activities:

- It is a competitive & selective B1-receptor antagonist which is attributable to the d-enantiomer

- It has mild vasodilating properties, possible due to an interaction with the L-arginine/nitric oxide pathway

Nebivolol reduces heart rate & blood pressure at rest & during exercise. In healthy volunteers it has no significant effect on maximal exercise or endurance. An in-vitro and in-vivo experiment in animals showed that nebivolol has no intrinsic sympathomimetic activity and at pharmacological doses has no membrane stabilizing effect. It is also devoid of alpha-adrenergic antagonism at therapeutic doses[8].

3. Materials and method

3.1 Materials

Amlodipine besylate was generously supplied as a gift sample by M/s Pravin Laboratories (P) Ltd, (Moje-Jolwa,Gujarat), Nebivolol Hydrochloride, M/s Cadila Pharmaceuticals (Ankleshwar, Gujarat). Lactose, M/s Dynamix India (Baramati, M.S.), Starch, M/s Tirupati Starch and Chemicals Ltd (Dhar, M.P.), and Microcrystalline cellulose (MCC) JRS Pharma (Rosenberg, Germany), All other chemicals were of analytical reagent grade and were used as received.

3.2 Formulations of S-(-)- Amlodipine besylate and Nebivolol Hydrochloride Tablet

The granules of microcrystalline cellulose-starch and lactose-starch were prepared in the ratio as shown in (Table 1). To arrive at an optimal formulation, preliminary data on various derived characters and physical characters of tablets were generated (Table 2). The granules were lubricated with magnesium stearate (0.5%) and talc (1%), and compressed using a Cadmach make double rotary single punch, with oval shaped punches. The drugs S-(-)- amlodipine besylate and nebivolol hydrochloride were added at the lubrication stage. The tablets had an average weight of 125 mg and each tablet contained 2.5 mg of S-(-)- amlodipine besylate and 5.0 mg nebivolol hydrochloride.

3.3 Evaluation

The tablets of S-(-)- amlodipine besylate and nebivolol hydrochloride was prepared by wet granulation technique and evaluated for preformulation and post formulation parameters, such as hardness, friability, disintegration, content uniformity and in vitro release profile.
Bulk and Tapped Density (Hausner's Ratio and Carr's Compressional Index):
The density parameters were determined using 10.0 g of each material in a 25 mL graduated cylinder (n = 3) (Electrolab Tap density tester USP: ETD-1020). The values were used to calculate Hausner's Ratio and Carr's Compressional Index[9,10].

3.4 Flowability

The flow properties of the sample were evaluated by the dynamic flow determination. The analysis was performed 3 times with 10.0 g of each sample[11].

3.5 Solubility Study of S-(-)- Amlodipine besylate and Nebivolol Hydrochloride

Maximal solubility of S-(-)- amlodipine besylate and nebivolol hydrochloride in various physiological pH (pH 1.2, pH 4.0, pH 6.8 Phosphate buffer, pH 7.4 Phosphate buffer), was studied. Excess amount of S-(-)- amlodipine besylate and nebivolol hydrochloride, respectively, was taken in 10 ml of the above media, in boiling test tube, and dissolved in triplicates by sonication. The maximal solubility of S-(-)- amlodipine besylate and nebivolol hydrochloride in each medium, wad determined at different time intervals (0, 15 and 60 min) after filtering the content of each test tube by Whatman filter paper, the S-(-)- amlodipine besylate and nebivolol hydrochloride content was determined spectrophotmetrically at 239 nm and 281 nm respectively.

3.6 In vitro Dissolution Study of S-(-)- Amlodipine besylate and Nebivolol Hydrochloride Tablet

The optimized formulation WMS 3 [Wet granulation microcrystalline cellulose:starch blend (50:50)], was selected and for in vitro dissolution study in USP XXIV dissolution apparatus type II at 37 ± 0.5°C and at 75 rpm, using 500 ml of dissolution media pH 1.2. The dissolution profile was compared with that of marketed preparation. The results recorded in Table 3.

3.7 Content uniformity

Content uniformity of the optimized batches was also studied, using HPLC, Shimadzu (model:LC – 2010HT). The results noted in Table 3.

3.8 Stability Studies

The selected formulation (WMS 3) was studied for accelerated stability studies as per the ICH guidelines[12]. The optical rotation of S-(-)- amlodipine besylate in the formulation was measured using Polarimeter, Perkin Elmer (model: 341).

4. Results and discussion

The studies revealed that S-(-)- amlodipine besylate and nebivolol hydrochloride has very poor flow property, and a very good compressibility. Microcrystalline cellulose:starch blend gave the satisfactory Carr's index (15.90), Hausner's ratio (1.189) and angle of repose (27˚). The blend appeared to be the better alternative as it had better compressibility and flow property as compared to lactose:starch blend. Results revealed that 5% starch paste gave optimized results, in terms of hardness and friability.

Experiments with solubility study of S-(-)- amlodipine besylate and nebivolol hydrochloride in various physiological pH, revealed that S-(-)- amlodipine besylate and nebivolol hydrochloride is soluble in pH 1.2. Hence pH 1.2 was the ideal dissolution media, to study in vitro release profile of S-(-)- amlodipine besylate and nebivolol hydrochloride. The optimized batch of S-(-)- amlodipine besylate and nebivolol hydrochloride tablet formulation WMS 3 was studied for the content uniformity and in vitro release profile in the above media. The results indicated improved dissolution profile of the formulated S-(-) - amlodipine besylate and nebivolol hydrochloride tablet (WMS 3) as compared with the available marketed preparations. The content uniformity was found to be NLT 85% and NMT 115%. The accelerated stability studies as per ICH guidelines revealed no change in optical rotation of S-(-)- amlodipine besylate.

5. Conclusions

The formulation and dissolution profile of formulation WMS 3 was encouraging. The trial conducted with consecutive three batches revealed RSD below 2%, indicative of insignificant batch-to-batch variation. The formulation showed improved dissolution as compared to the marketed preparation. Thus the formulation of S-(-)- amlodipine besylate and nebivolol hydrochloride using microcrystalline cellulose:starch blend would be cost effective and dissolution media pH 1.2 would the ideal media for conducting dissolution studies.

Table 1. Formulation of S-(-)- amlodipine besylate and nebivolol hydrochloride by wet granulation techniques.

Batch Parameters	WLS 1	WLS 2	WLS 3	WMS 1	WMS 2	WMS 3
Lactose:Starch	80:20	60:40	40:60	-	-	-
MCC:Starch	-	-	-	80:20	60:40	40:60
Starch Paste	5%	5%	5%	5%	5%	5%
Crosscarmellose Sodium (%w/w)	1%	1.5%	2.0%	-	-	-
Hardness (kg/cm^2)	5.0	3.0	2.0	7.0	5.0	4.0
Friability (%w/w)	0.22	0.42	0.8	0.05	0.11	0.16
Disintegration (sec)	190	125	82	42	62	71

Each tablet contains 2.5 mg S-(-)- amlodipine besylate and 5.0mg nebivolol hydrochloride, each tablet weighs 125 mg.
WLS- Wet granulation lactose:starch blend, WMS-Wet granulation microcrystalline cellulose:starch blend.

Table 2. Effect of diluent on derived properties of S-(-)- amlodipine besylate and
nebivolol hydrochloride granules

Batch Parameters	Drug without diluents	WLS 1	WLS 2	WLS 3	WMS 1	WMS 2	WMS 3
Bulk density (g/ml)	0.562	0.489	0.389	0.346	0.419	0.409	0.386
Tap density (g/ml)	0.637	0.642	0.549	0.506	0.468	0.470	0.459
Carr's index	11.77	24	29	31.60	10.42	12.97	15.90
Hausner's ratio	1.133	1.312	1.411	1.462	1.116	1.149	1.189
Angle of repose (^{o})	>50	26	29	33	17	24	27

WLS- Wet granulation lactose: starch blend, WMS-Wet granulation microcrystalline cellulose:starch blend

Table 3. Content and dissolution profile of S-(-)- amlodipine besylate and nebivolol
hydrochloride tablet.

Product	Content (%w/w)	Dissolution media	% Released 45 (min)
WMS 3		pH 1.2	
S-(-)- amlodipine besylate	100.11	-	100.03
Nebivolol Hydrochloride	99.92	-	99.79
Marketed preparation		pH 1.2	
S-(-)- amlodipine besylate	98.72	-	91.44
Nebivolol Hydrochloride	97.65	-	92.31

WMS 3: Wet granulation microcrystalline cellulose:starch blend (40:60).

References

[1] P. K. Whelton, J. He, L. J. Appel, Primary prevention of hypertension: Clinical and public health advisory from the National high blood pressure education programme, JAMA **288**, 1882 (2002).

[2] J. Luksa, D. Josic, M. Kremser, Z. Kopitar, S. Milutinovic, J Chromatogr Biomed Sci Appl., **703**, 185 (1997).

[3] J. Luksa, D. Josic, B. Podobnik, B. Furlan, M. Kremser, J Chromatogr Biomed Sci Appl., **693**, 367 (1997).

[4] H. Laufer, M. Leitold, Chirality **6**, 531 (1994).

[5] M. Ohmori, M. Arakawa, K. Harda, H. Takasali, S. Hifumi, I. Miyamori, A. Fujimura, Am J Ther. **10**, 29 (2003).

[6] E. Benson, J. Webster, Br J Clin Pharmacol. **39**, 578 (1995).

[7] P. A. Meredith, H. L. Eliott, Clin pharmacokinetics. **22**, 22 (1992).

[8] P. L. Munson, Principles of Pharmacology Basic Concepts and Clinical applications, Chapman and Hall, New York, 544, (1995).

[9] H. H. Hausner, Int J Powder Mettall. **3**, 7 (1967).

[10] R. L. Carr, Evaluating flow properties of solids, Chem Engineer **72**, 163 (1965).

[11] J. C. Guyot, P. Arnaud, P. Becourt, STP Pharma Pratiques, **5**, 482 (1995).

[12] ICH Harmonised Tripartite guidelines stability testing of new drug substances and products Q1 A (R2), 2003.