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Solubilized formulation and evaluation of Reloxifene for prevention and treatment of osteoporosis in postmenopausal women

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Abstract

An increasing number of new chemical entities emerging from drug discovery and reaching drug development are poorly or very poorly water soluble. Our study aimed at developing solubilized formulation and evaluation of insoluble drug raloxifene (RLX) with improved bioavailability for the treatment of osteoporosis in postmenopausal women. Preformulation studies were conducted to determine the physical characteristic of drug like bulk density, particle size and solubility. Solid dispersions were prepared by hot melt method by employing 6 different carriers and mixing ratios (1:1, 1:2, and 1:3). And selection of suitable complexing agent/ carriers and ratio was done on the basis of solubility. DSC studies were made to define the physical state of drug in the carrier and possible interaction of RLX- carrier complex. Compatibility of excipients with RLX- carrier complex was tested with regard to their taste, in order to increase the palatability of the RLX formulation. Totally 10 trial bathes of RLX formulation were conducted to select prototype formula, batch S0010 was selected as optimized formulation. The best formulation obtained was investigated by preliminary stability studies at fresh (stored at RT) and stressed (stored at 40ºC & 75% RH for 1 month) conditions and initial RLX formulation were compared with aged (1 month) one. No substantial changes in its stability were observed.

Keywords: Insoluble drug, Raloxifene (RLX), Solubilization, Osteoporosis, DSC.

Introduction

At the turn of the century, a woman's menopause signaled the last remaining years of her life. With the average life expectancy of women close to 80 years of age, women can now expect to live up to one-third of their lives in the postmenopausal state. With the menopause, a number of hormonal changes occur, the most notable of which is a reduction in estrogen levels that has been demonstrated to be major risk factor for the development of osteoporosis and cardiovascular disease [1, 2]. Osteoporosis is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture. The bone loss commences in the first year after menopause and is arrested within 6 years after the onset of menopause. The overall bone loss during this phase is estimated to be approximately 15% [3].

The use of solid dispersions of drugs in water- soluble carriers, in which the drugs are highly soluble, to increase the dissolution rate and bioavailability of poorly soluble drugs has been studied extensively [4,5]. This technique provides a means of reducing particle size to nearly a molecular level. As the soluble carrier dissolves, the insoluble drug is exposed to the dissolution medium as very fine particles for quick dissolution and absorption. As a part of a new drug development program, we have investigated the possible use of this technique to improve the bioavailability of a insoluble drug, reloxifene (RLX). Raloxifene, a selective estrogen receptor modulator, is approved for the prevention and treatment of postmenopausal osteoporosis [6] and available in the form of conventional dosage form (Tablet). Our investigation was designed to develop solubilized liquid oral solution of insoluble drug RLX for faster absorption with least potential for bioavailability problems.

Materials and methods

Raloxifene (Dr. Reddy's Laboratories), Citric acid (Sigma laboratories), Polyethylene glycol 400, PEG 4000 (Hi-tech chemicals). All other chemicals and reagents used were analytical grade.

Preformulation studies

In order to maximize the chances in formulating an acceptable, efficacious and stable product, preformulation testing was done with an investigation of physical and chemical properties of a drug substance alone and combined with excipients.

Determination of bulk density

An accurately weighed quantity of the RLX drug which was previously passed through sieve # 40, was carefully poured into graduated cylinder and the volume (V_o) was measured. Then the graduated cylinder was closed with lid, set into density determination apparatus**.** The density apparatus was set for 100 taps and after that the volume (V_f) was measured and continued till the two consecutive readings were equal. The bulk density was calculated using the following formula: Bulk Density = W/ V_o

Tapped Density = W/V_f

Where W = Weight of powder; V_o = Initial volume; V_f = final volume

Powder fineness of raloxifene

Powder finess was measured by weighing 1 gm of powde into 1000 ml beaker containing miili Q water and this was sonicated for 20 min and this solution was used as dispersant. The test solution was prepared by adding 200 mg of the sample into 30 ml stoppered test tube, 25 ml of dispersant was added and kept on cyclomixer for 2 min, than this was again sonicated for 30 min. Sample handling unit was filled with dispersant, and required stirring speed was set and stirred to eliminate any air bubbles and back ground was measured. Add the well dispersed sample drop wise into the sample dispersion unit until the obscuration falls in to the range of ideal.

Solubility

Solubility of RLX was checked in different solvents. Excess amount of RLX was taken in screw capped glass tube and 1 ml of solvent was added. The tube was shaken for 24 hrs in a shaking water bath and the drug solubility was determined.

Preparation of RLX: carrier complex

Solid dispersions of RLX in different carriers such as citric acid, poloxamer, povidon K 30, ascorbic acid, succinic acid, polyethylene glycol 4000 were prepared by the hot melt method with the intention of improving the dissolution properties of the drug. Different ratio's of drug and carrier were weighing accurately into a beaker, mixed thoroughly and was allowed to melt at higher temperatures approximately 100^o C. The molten mixture was than poured onto a cold Petri dish and kept in refrigerator for rapid cooling in order to obtain a solid dispersion.

Thermal analysis of the sample by DSC

Thermal analysis was carried out with the help of DSC [7]. The temperature was elevated from ambient (25^oC) to 300^oC at the heating rate 10^oC/ min under the nitrogen environment**.** 5mg of the samples were weighed and subjected for DSC.

RLX: carrier complex - excipients taste evaluation

Each excipient used in the formulation was thoroughly mixed with the drug complex to increase the RLX: carrier - excipient molecular contact to the accelerate reaction if possible. Physical mixtures of RLX: carrier complex-excipients were tested for stability by physical observation. Compatibility of excipient with complex was tested with regard to their taste.

Development of raloxifene (RLX) formulation oral solution

An RLX oral solution was prepared by dissolving the RLX – Carrier complex in suitable solubilizing agents, and surfactants were added to obtain a clear solution without any precipitation and turbidity. To improve the Palatability of the formulation other additives like sweetening agents, flavors were added. Preservative was added to prevent any microbial contaminations.

The complex containing RLX and citric acid was dissolved in polyethylene glycol for batches S001 and S002; the RLX complex was dissolved in glycerol for batches S009 and S0010 and kept for sonication for 1 hour. Sucrose in batch S001 and sodium saccharine in batch S002, S009 and S0010 was added respectively along with sodium benzoate and pineapple flavor**.** The two mixtures were mixed thoroughly with continuous stirring and final volume was made.

For batches S003, S004, S005, S006, S007, S008 the fooling procedure was followed. RLX was heated with povidon K 30 along with polyethylene glycol. To this mixture PEG 400 and glycerol were added. Poloxamer was dissolved in small quantities of water and added to batches S003. S004, S005, S007 and S008 and gelucire was added to batch S006.To the above batches sodium benzoate and pineapple flavor was added and final volume was made with water. The quantities

TABLE: 1 COMPOSITION OF TRIAL BATCHES

	(Quantity) grams									
Ingredients	**Batch S001**	**Batch S002**	**Batch S003**	**Batch S004**	**Batch S005**	**Batch S006**	**Batch S007**	**Batch S008**	**Batch S009**	**Batch S0010**
RLX			0.06	0.06	0.06	0.06	0.06	0.06		
RLX Complex	0.12	0.12							0.12	0.12
Polyethylene glycol-400	0.83	0.83	0.32	0.32	0.32	0.32	0.32	0.6		
Glycerol			0.83	1	2.5		1.6	1.6	2.5	4
Polyethylene glycol			0.2	0.2	0.2	0.2	0.2	0.2		
Sucrose	2.16									
Sodium saccharine			0.016	2.6		0.025	0.025	0.03	0.04	0.025
Aspartame		0.025								
Poloxamer			0.2	0.25	0.25		0.25	0.25	0.2	0.16
Gelucire						0.28				
Polysorbate 80										
Povidon K 30			0.06	0.06	0.06	0.06	0.06	0.06		
Sodium Benzoate	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016
Pineapple flavor	0.0016	0.0016	0.0016	0.0025	0.0025	0.003	0.025	0.0025	0.003	0.003
water	up to 5ml	up to 5ml	up to 5ml	up to 5ml	up to 5ml	up to 5ml	up to 5ml	up to 5ml	up to 5ml	up to 5ml

RLX Complex = RLX and Citric acid – 1 in 1:1 ratio of additives added in batches (S001-S0010) were shown in the Table: 1.

Physical appearance:
The developed RLX liquid oral solution was evaluated for physical appearance like color, clarity, turbidity, precipitation, crystal growth, palatability, viscosity and pH.

Drug content (assay):
The drug content of liquid oral solution was determined by using 0.001 N HCl as blank at 284 nm in UV spectrophotometer.

Preliminary stability studies
Preliminary stability of the best formulation obtained was investigated by studying its Physical characteristics and Drug content after storage at stressed conditions. Solution was stored at 40^{o}C & 75% RH for a period of 1 month. The Physical characteristics and drug content of the fresh (stored at room temperature) and stressed preparations were compared to evaluate the stability and effect of aging.

Results and Discussion

The physicochemical characteristics of a drug relevant to its biological activity are determined at the preformulation stage of drug development. Factors such as bulk density, particle size, solubility were determined and the preformulation parameters were given in the Table: 2.

Table 2: Physical Characteristics of Rlx

Bulk density Before tapping After tapping	0.22 g/ml 0.53 g/ml
Particle size by Malvern	10 % less than 11 µm 50% less than 46 µm 90% less than 114 µm
Solubility	Freely soluble in dimethyl sulfoxide Very slightly soluble in methanol Insoluble in water

Standard calibration curves of RLX in 0.001 N HCl was given in Figure 1**.** The beer's law was obeyed in the concentration range of 2.5 – 15ug/ml. The linearity coefficient in each case was greater than 0.993.

To improve the dissolution performance**,** the drug was formulated into solid dispersion using 6 different carriers and mixing ratios. As intermolecular association of drug with the carrier led to increased effective surface area of drug in the solid dispersion, resulting in solubility enhancement of the drug.

Solid dispersions of RLX were prepared by hot melt method by employing various carriers to improve dissolution properties of the drug. And selection of suitable complexing agent/carrier was done on the basis of solubility of complex in different solvents; the data is summarized in Table 3.

Table: 3 selection of complexing agent/carrier on basis of solubility

Serial No	Drug	Carrier	Water	Polyethylene glycol-400	Glycerol 80%	Propylene glycol
1	RLX	Citric acid	Soluble	Soluble	Soluble	Soluble
2	RLX	Poloxamer	Soluble	Insoluble	Insoluble	Soluble
3	RLX	Povidon K30	Insoluble	Insoluble	-	-
4	RLX	Ascorbic acid	Insoluble	Soluble	Insoluble	Insoluble
5	RLX	Succinic acid	Insoluble	Insoluble	Insoluble	Soluble
6	RLX	Polyethylene glycol-4000	Insoluble	Insoluble	-	-

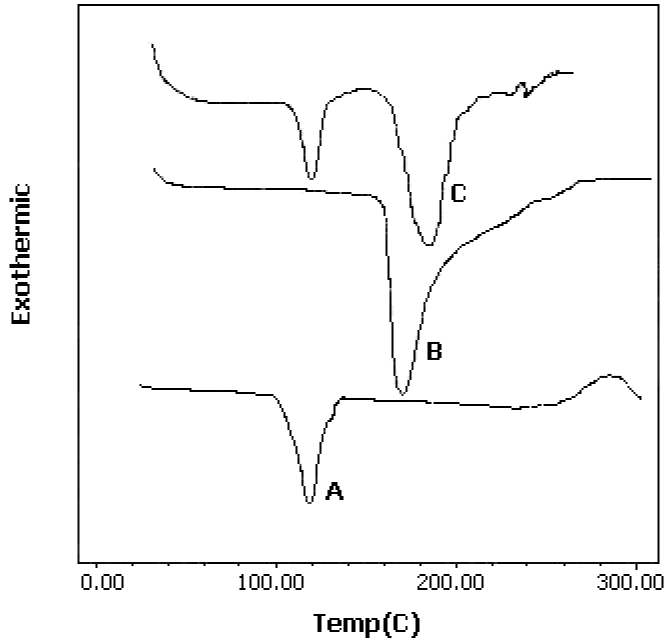
The complex containing RLX and citric acid carrier are completely soluble in all solvents without any precipitation or turbidity, and form a clear, bright yellow colored solution in water.
Solid dispersions of carrier and drugs at different weight ratios were prepared by hot melt method with an aim to improve the solubility of postmenopausal osteoporosis drug RLX. RLX and carrier at different ratio, namely 1:1, 1:2, 1:3 were used for the preparation of solid dispersion. The RLX : carrier ratio (1:1) were clear, bright yellow color after 10 min in water and clear, bright yellow color after sonication for 20 min in aqueous solvents, where as 1:2 were insoluble and 1:3 were pale, light yellow turbid solution in water. Among these RLX : carrier ratios, 1:1 ratios were taken for dosage form formulation as these complex were completely soluble and forms clear, bright yellow colored solution both in water and other solvent in comparatively less time than any other complexes.

The DSC thermograms of the RLX, carrier and RLX- carrier complex were made to define the physical state of the RLX in the carrier and the possibility of interaction between the RLX and the carrier [7]. DSC studies indicated no interaction between RLX and carrier. Raloxifene showed (Fig: 2) a melting temperature 118º C and similar results were obtained when complex of 1:1 were subjected to thermal analysis. This data suggest that RLX and carrier were neither dissolved nor formed a covalent linkage with the carrier. Fig: 2 provides the thermal behavior of the pure RLX, carrier and combination of RLX- carrier in a ratio approximately representing the formula.

Table: 4 Taste of RLX complex in selected excipients

Drug complex+ Excipients	Citric Acid	Glycerol	Propylene Glycol	Sucrose	Sodium Saccharine	Aspartame	Poloxamer	Geluire	Polysorbate 80	Povidon K30	Sodium Benzoate
Taste	Bitter	Sweet	Acride	Sweet	Sweet, Metallic	Sweet	Tasteless	Tasteless	Bitter	Tasteless	Saline

Figure: 1 Differential Scanning Calorimetry (DSC)



A: RLX, B: CITRIC ACID, C: RLX-CITRIC ACID COMPLEX

Compatibility of excipient with complex was tested with regard to their taste. Physical mixture of the RLX complex and excipients were made, results was showed in Table: 4**.** The drug complex containing raloxifene and citric acid was very bitter tasting; hence its compatibility with other excipients was studied with regard to taste, in order to increase the palatability of the RLX oral solution.

Trial batches (S001-S0010) of RLX oral solution was conducted and organoleptic properties were evaluated to select prototype formula. Among these ten trial batches, RLX oral solution of batch S0010 was selected as optimized formulation because**,** the taste of this batch was much better with pineapple odor than the other trial batches, and the final oral solution was clear, bright yellow colored without any precipitation or turbidity and crystal growth. Oral solution (S0010) showed viscosity of 160-168 cps and pH was 3.

In the initial stage, different excipients and their combinations were tried according to the literature report or trial and error method to achieve the desired palatable solution. Once a prototype formulation with an acceptable taste was obtained, it was kept for preliminary stability studies. Preliminary stability of the best RLX formulation obtained was investigated by studying its physical characteristics and Drug content after storage at stressed conditions. Solutions were stored at 40^{o}C & 75% RH and at refrigerator temperature for 1month.Pysical characteristics and drug content of the fresh (stored at room temperature) and stressed preparations were compared to evaluate the stability and effect of aging. The results were summarized in Table: 5.

Table: 5 Stability data of prototype formulation (S0010)

Parameters Physical stability	Initial	15 days			1 month		
		Room Temp	40ºC ±2ºC/ 75%RH +6%	Refrigerator temp	Room Temp	40ºC ±2ºC/ 75%RH +6%	Refrigerator temp
Color	Bright yellow	No change	No change	No change	No change	No change	No change
Clarity	Clear	No change	No change	No change	No change	No change	No change
Turbidity	No	No	No	No	No	No	No
Precipitation	No	No	No	No	No	No	No
Crystal growth	No	No	No	No	No	No	No
Taste	Sweet	No change	No change	No change	No change	No change	No change
Odor	Pineapple	No change	No change	No change	No change	No change	No change
Viscosity	160-168 cps	162-169 cps	161-164 cps	161-164 cps	161-164 cps	160-166 cps	164-169 cps
pH	3	3	3	3	3	3	3
Assay	102.30%	101.90%	100.24%	104.70%	99.80%	101.30%	100.20%

The physical observations and drug content of the aged formulation (1 month at 40^{o}C and 75% RH) was compared with the initial formulation. No substantial change in physical characteristics, and drug content was observed between the stressed and control preparations

Conclusion

The present study was undertaken to develop solubilized formulation of insoluble postmenopausal osteoporosis drug RLX. The final formulation is robust once and stable. The developed RLX formulation was expected to have better bioavailability and patient acceptability as compared to conventional dosage forms. However this needs to be confirmed by bioavailability studies.

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