ARTÍCULO ORIGINAL

Stability study of Raloxifene tablets

Estudio de estabilidad realizado en tabletas de Raloxifeno

Dr. C. Jorge Enrique Rodríguez Chanfrau, MSc. Iverlys Díaz Polanco, Téc. Marilyn López Armas

Centro de Investigaciones y Desarrollo de Medicamentos. La Habana, Cuba.

RESUMEN

Introducción: el raloxifeno es un modulador selectivo del receptor estrogénico perteneciente a la familia de los benzotiofenos. Varios ensayos clínicos han mostrado que el raloxifeno reduce la pérdida del hueso en la espina dorsal y puede aumentar la masa de hueso en ciertos sitios.
Objetivo: determinar la estabilidad física y química de las tabletas de raloxifeno.
Métodos: se prepararon tres lotes a escala piloto de 5 kg cada uno. Se realizaron estudios de disolución *in vitro*, estabilidad química, fotoestabilidad y humedad. Se colectaron las muestras al tiempo 0, 1, 2, 3 y 6 meses para el estudio de estabilidad acelerado y a tiempo 0, 6, 12, 18 y 24 meses para la estabilidad por vida de estante. Se determinó la estabilidad química aplicando un método de análisis por cromatografía líquida de alta resolución, desarrollado y validado previamente.
Resultados: en estudio de estabilidad acelerada, el ensayo de disolución *in vitro* mostró disolución del ingrediente activo en concentraciones mayores al 90 %, mientras que la concentración se mantuvo entre el 90 y 110 %. Los estudios en condiciones de humedad afectaron la estabilidad química del medicamento.
Conclusiones: todos los lotes de la formulación de tabletas de raloxifeno resultaron estables por 24 meses en los envases estudiados, almacenados a la temperatura de 32 ± 2 ºC y protegidos de la humedad. Las tabletas presentan una buena disolución *in vitro* durante los 24 meses.

Palabras clave: raloxifeno, estabilidad, disolución *in vitro*, tabletas.

ABSTRACT

Introduction: Raloxifene is a selective estrogen receptor modulator from the benzothiophene family. Several clinical trials have shown that raloxifene reduces bone loss rate in the spinal column and may increase bone mass at certain sites.
Objective: to determine the physical and chemical stabilities of raloxifene tablets.
Methods. three pilot scale batches of 5 kg each were prepared. In vitro dissolution, chemical stability, photostability and humidity studies were carried out. Samples were collected at 0, 1, 2, 3 and 6 months for the accelerated stability study and at 0, 6, 12, 18 and 24 months for the shelf life stability study. Chemical stability was determined using high performance liquid chromatography analytical method, which was developed and validated prior to the study.
Results: in the accelerated stability study, the percentages of dissolved drug were more than 90 % and drug content porcentages were between 90 % and 110 %. Humidity conditions affected the chemical stability of the tablets. Conclusions: All raloxifene tablet batches formulations were stable for 24 months in the studied containers stored at 32 ± 2 ºC and waterproof. *In vitro* drug release dissolution showed good results for 24 months.

Key words: Raloxifene, stability, *in vitro* dissolution, tablets.

INTRODUCTION

Raloxifene is a selective estrogens receptor modulator, which belongs to the benzothiophene family. Estrogen receptor binding causes acivation and blocking of certain strogenic pathways. Thus, raloxifene is an estrogen agonist/antagonist, commonly referred to as a selective estrogen receptor modulator. The results of several clinical trials have shown that raloxifene decreases bone resorption and reduces biochemical markers of bone turnover to the premenopausal range.[1-6]

The raloxifene is supplied in a tablet dosage form for oral administration. Each tablet contains 60 mg of raloxifene HCl, which is the molar equivalent of 55.71 mg of free base. It is indicated to reduce the risk of invasive breast cancer in postmenopausal women with osteoporosis.[4,5]

The physicochemical and biological properties of the active substance are the most important characteristics when the generic pharmaceutical dosage form was developed. At the same time, it is necessary to develop an analytical method validated according to international literature.[7,8]

The most common reactions observed in pharmaceutical dosage forms are hydrolysis, dehydration, isomerization, oxidation, photodegradation, and some specific interactions with formulation components (excipients and their impurities).[9] Evaluating the stability parameters is useful to drug formulation and is helpful to know about the storage conditions. In long-term studies, the formulation stability will dictate the shelf life of the marketed product.[10-13]

The objective of this work was to evaluate the raloxifene tablet stability.

METHODS

Three pilot scale batches of 5 kg, equivalent to 27778 tablets each one (08001, 08002 and 08003) were prepared. Raloxifene hydrochloride (BTP Pharmaceutical Co. Lim.) of pharmaceutical quality was used as active pharmaceutical ingredients.[14]

Polyvinylpyrrolidone (Kollidon K 25®) (Germany), Lactose (China), Sodium Starch Glicolate (Brazil), Polysorbate 80 (Germany), Microcrystalline Cellulose (Avicel PH 101) (Germany) Magnesium Estearate (China) were used in the preparation of the tablet core. The coating formulation consisted of Policoat (YS 1-7003) polymers and Tartracine suspended in ethanol.

Raloxifene hydrochloride tablets of 60 mg were prepared by wet granulation method. All ingredients were mixed (Planetary granulation equipment model HOBART, Germany) and manufactured using a rotary tablet machine (MANESTY F3, Italy) equipped with flat, bevelled edge, punches, 8 mm in diameter, to an average hardness of 5 ± 1 kgf and an average weight of 180 ± 10 mg. Each batch was analyzed and subsequently packaged in PVC and aluminium blisters and high-density polyethylene plastic bottle.

Analytical assay

Chemical stability was determined by high performance liquid chromatography (HPLC) method according to the criteria proposed by CECMED.[13] The analyses were performed using the HPLC KNAUER (Germany) equipment under the following conditions: C18 column, mobile phase (methanol, water and trietanolamine in the ratio of 70:30:0.1 v/v), injection volume of 20 µL, flow rate of 1.5 mL/min and λ= 286 nm, using a previously validated method.[15]

Determination of *in vitro* dissolution of raloxifene tablets

Dissolution process was carried out following the U.S.P general method.[14] PHARMA TEST, model PTW S3C model (Germany) dissolution apparatus with paddles was used. An agitation rate of 100 rpm for 60 min, 900 mL of 0.1 mol/L hydrochloric acid solution as dissolution medium and 37 ± 0.1 ºC temperature were the experimental conditions. All samples were added to the medium, withdrawn and filtered by 0.45 µm pore diameter filter. The analysis of dissolved raloxifene was according above mentioned the HPLC method .

Stability study

Tablets representing the three batches were placed in containers for accelerated (40 ± 2 °C/75 ± 5 % RH for 6 months) and long-term (30 ± 2 °C/70 ± 5 % RH for 24 months) stability studies. Samples were collected at 0, 1, 2, 3 and 6 months (accelerated stability studies) and at 0, 6, 12, 18 and 24 months (long-term stability studies) for chemical stability and *in vitro* dissolution tests. Experimental results were expressed as means ± SD.

Photostability and humidity studies

Samples of each batch were placed in sealed clear glass containers and exposed to light source during 90 days following the ICH test conditions for photostability test.[16] On the other hand, samples of each batch were placed into a humidity chamber at 84, 92 and 98 % during 180 days. At the end of both studies, the samples were analyzed. Experimental results were expressed as means ± SD.

RESULTS

Table 1 shows the quality control results of each batch before packing. These results comply with the quality control parameters indicated in the analysis methods (Rodríguez Chanfrau JE, López Armas M. Raloxifeno tabletas. Técnica de fabricante Nº PT 11001. CIDEM. 2011)

Table 1. Initial analytical results

Parameters	Batches			Acceptance limits
	08001	08002	08003	
Organoleptic	Respond	Respond	Respond	Film coated tablet, beveled of pale yellow color
Dissolution (%)	94.2 ± 1.47	94.8 ± 2.14	94.7 ± 1.21	No less than 80 % (*Q*) of the labeled amount of drug content is dissolved in 60 min
Drug content (%)	99.6 ± 1.25	98.3 ± 2.01	99.1 ± 0.75	Between 90.0 % and 110.0 % of drug content

Table 2 shows the results of accelerated stability studies and table 3 shows the results of long-term stability studies in both studied containers. The results showed that the raloxifene tablets fulfilled the quality specifications after 6 months and 24 months, respectively. Similar results were observed for photostability studies after 90 days (table 4).

The humidity conditions that were used affected the chemical stability of raloxifene tablets (Fig.). Serious affects were observed when the tablets were packed in high density polyethylene plastic bottle.

Table 2. Analytical results of the batches under accelerated stability conditions

Dissolution (%)					
Sample	Initial	1 months	2 months	3 months	6 months
08001 B	94.2 ± 1.47	94.5 ± 0.85	93.9 ± 2.65	94.8 ± 2.99	94.1 ± 0.54
08002 B	94.8 ± 2.14	94.2 ± 2.77	94.6 ± 1.18	94.3 ± 2.82	94.7 ± 1.95
08003 B	94.7 ± 1.21	94.5 ± 0.71	95.2 ± 2.44	94.1 ± 2.90	94.4 ± 1.79
08001 F	94.2 ± 1.47	94.3 ± 1.75	94.8 ± 1.48	93.8 ± 1.17	93.5 ± 1.27
08002 F	94.8 ± 2.14	93.8 ± 2.21	94.1 ± 1.20	94.6 ± 0.98	94.0 ± 1.79
08003 F	94.7 ± 1.21	94.5 ± 3.62	95.2 ± 1.86	94.7 ± 1.75	95.2 ± 1.83
Drug content (%)					
Sample	Initial	1 months	2 months	3 months	6 months
08001 B	99.6 ± 1.25	99.7 ± 0.99	99.6 ± 1.77	99.4 ± 1.66	99.0 ± 2.95
08002 B	98.3 ± 2.01	98.0 ± 1.14	98.2 ± 1.51	98.6 ± 1.98	98.5 ± 1.15
08003 B	99.1 ± 0.75	99.9 ± 2.15	99.7 ± 2.75	99.6 ± 1.65	99.5 ± 2.01
08001 F	99.6 ± 1.25	99.5 ± 1.48	99.3 ± 0.55	99.2 ± 2.18	99.3 ± 1.78
08002 F	98.3 ± 2.01	98.5 ± 1.94	99.3 ± 3.18	98.5 ± 0.41	98.9 ± 3.01
08003 F	99.1 ± 0.75	99.2 ± 1.55	99.9 ± 1.96	99.7 ± 1.06	99.4 ± 1.23

B: batch packed in PVC and aluminium blisters;
F: batch packed in high-density polyethylene plastic bottle.

Table 3. Analytical results of the batches under long-term stability conditions

Dissolution (%)					
Sample	Initial	6 months	12 months	18 months	24 months
08001 B	94.2 ± 1.47	93.0 ± 1.21	93.7 ± 0.75	97.0 ± 3.43	96.1 ± 1.83
08002 B	94.8 ± 2.14	95.2 ± 0.98	95.6 ± 0.72	96.3 ± 2.40	94.2 ± 2.95
08003 B	94.7 ± 1.21	96.5 ± 0.82	93.2 ± 1.05	93.1 ± 2.25	95.4 ± 1.63
08001 F	94.2 ± 1.47	94.2 ± 1.87	93.8 ± 1.87	95.8 ± 1.47	93.9 ± 2.07
08002 F	94.8 ± 2.14	93.4 ± 2.14	92.1 ± 1.51	97.0 ± 1.94	95.0 ± 2.95
08003 F	94.7 ± 1.21	94.8 ± 2.64	94.2 ± 2.25	95.7 ± 1.47	95.6 ± 1.94
Drug content (%)					
Sample	Initial	6 months	12 months	18 months	24 months
08001 B	99.6 ± 1.25	99.3 ± 1.09	98.7 ± 1.55	99.2 ± 1.96	99.0 ± 0.75
08002 B	98.3 ± 2.01	98.0 ± 0.97	97.9 ± 1.02	97.7 ± 2.14	97.9 ± 1.52
08003 B	99.1 ± 0.75	99.2 ± 0.25	98.6 ± 2.31	98.9 ± 2.05	98.9 ± 1.94
08001 F	99.6 ± 1.25	99.5 ± 1.68	99.2 ± 1.92	99.1 ± 0.95	99.2 ± 1.88
08002 F	98.3 ± 2.01	98.2 ± 0.14	98.0 ± 0.44	97.8 ± 1.87	97.9 ± 1.33
08003 F	99.1 ± 0.75	98.8 ± 1.64	98.9 ± 1.51	98.5 ± 1.96	98.0 ± 2.05

B: batch packed in PVC and aluminium blisters;
F: batch packed in high-density polyethylene plastic bottle.

Table 4. Results of the photostability study

Sample	Dissolution (%)		Drug content (%)	
	Initial	90 days	Initial	90 days
08001 B	94.2 ± 1.47	94.5 ± 1.87	99.6 ± 1.25	99.2 ± 0.65
08002 B	94.8 ± 2.14	94.2 ± 1.47	98.3 ± 2.01	97.8 ± 1.42
08003 B	94.7 ± 1.21	94.3 ± 1.17	99.1 ± 0.75	98.9 ± 0.99
08001 F	94.2 ± 1.47	93.8 ± 1.47	99.6 ± 1.25	99.2 ± 1.55
08002 F	94.8 ± 2.14	94.5 ± 1.64	98.3 ± 2.01	97.9 ± 0.23
08003 F	94.7 ± 1.21	94.8 ± 1.60	99.1 ± 0.75	98.6 ± 1.96

B: batch packed in PVC and aluminium blisters;
F: batch packed in high-density polyethylene plastic bottle.

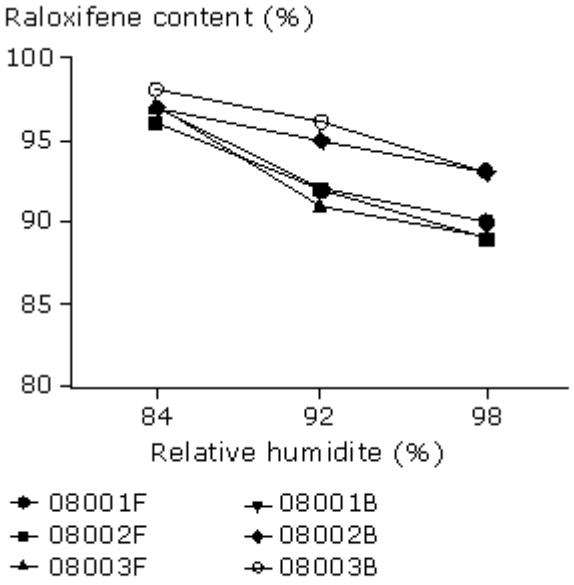

Fig. Analitical results up to 180 days under different humidity conditions.

A statistical comparison among the containers used in different relative humidity conditions showed that there was not significant differences to 84% (p= 0.3469). However, when the content of humidity is increased there was a statistically significant difference in the raloxifene content (p= 0.0014 and p = 0.0006 for 92 % and 98 %, respectively). However, during this study changes in the organoleptic characteristics of the samples were not observed.

DISCUSSION

The main factors that affect the drug stability are the temperature, pH, relative humidity of the environment, light, oxygen, physical form and particle size of the drug and excipients.[9] Stability studies provide information about some factors which influence the stability of a pharmaceutical preparation under study.

In our accelerated stability study, the percentages of dissolved drug were higher than 90 % (Q 80 % in 60 min) and drug content porcentages were between 90 % and 110 % in both containers. These results demonstrated that the temperature does not affect the product quality under the selected working conditions. Similar results were obtained in the photostability study. These results were similar to those reported for *Srinivas* et al.[17] which demonstrated that degradation was not observed in Raloxifene HCl sample when subjected to stress conditions such as light and heat.

Humidity conditions affected the chemical stability of the tablets. Raloxifene content decreased when the percentage relative humidity increased. The greatest effect was observed when the tablets were packed in high-density polyethylene plastic bottle (approximately 2 % at 84 % HR, 7 % at 92 % HR and 9 % at 98 % HR) compared to the tablets packed in PVC (approximately 1 % at 84 % HR, 3 % at 92 % HR and 7 % at 98 % HR). Therefore, the humidity has an impact on the quality product regardless of the type of container used. This parameter should be controlled during the storage of the product.

The most common reaction observed in pharmaceutical products is the hydrolysis. The relative humidity of the atmosphere affects the development of this process which is the main mechanism of drug degradation in solid state and in solution.[18,19]

Functional groups derived from carboxylic acid are common in pharmaceuticals and it is well-known that drug easily suffers degradation by hydrolysis when water is present, due to the existence of hydrogen or hydroxyl ions that likely catalyzes hydrolytic reactions.[18]

Raloxifene is a polyhydroxylated non-steroidal compound with a benzothiophene core that presents a carboxylic group in their chemical structure. This chemical group suffers the typical reactions of the carboxylic acids in presence of water. Four impurities ([2- (4-hydroxyphenyl)- 6-hydroxybenzo[b]thien-3-yl][4-[2-(1-piperidinyl)ethoxy]phenyl]methanone N-oxide; 6-Methylsulfonyloxy-2-[(4-methylsulfonyloxy)phenyl] benzothiophene; 6-Methylsulfonyloxy-2[(4-methyl sulfonyloxy)phenyl]-3-[[4(2-piperidinyl)ethoxy]benzoyl]benzothiophene Hydrochloride and 4-[2-(piperidinyl)ethoxy]benzoic acid) have been isolated during the degradation processes of raloxifene according the report of *Srinivas* et al.[17] In this study the degradation products could not be determined, but the results of humidity study indicated that in presence of water the active principle suffers a degradation process which is more prominent when the tablets are packed in high-density polyethylene plastic bottle.

On the basis of these present results, the raloxifene tablets formulation was stable with optimum *in vitro* drug release during 24 months in the studied containers, stored at 32 ± 2 ºC and protected from humidity.

REFERENCES

1. Delmas PD, Bjarnason NH, Mitlak BH. Effects of raloxifene on bone mineral density, serum cholesterol concentrations, and uterine endometrium in postmenopausal women. N Engl J Med.1997;337:1641-7.

2. Ettinger B, Black DM, Mitlak BH, Knickerbocker RK, Nickelsen T, Genant HK, Christiansen C, Delmas PD, Zanchetta JR, Stakkestad J, Gluer CC, Krueger K, et al.

Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene: results from a 3-year randomized clinical trial. Multiple Outcomes of Raloxifene Evaluation (MORE) Investigators. JAMA. 1999;282(7):637-45.

3. Lippman ME, Krueger KA, Eckert S, Sashegyi A, Walls EL, Jamal S, et al. Indicators of lifetime estrogen exposure: effect on breast cancer incidence and interaction with raloxifene therapy in the multiple outcomes of raloxifene evaluation study participants. J Clin Oncol. 2001;19(12):3111-6.

4. D'Amelio P, Muratore M, Tinelli F, Tamone C, Cosentino L, Quarta E, Calcagnile F, Carlo Isaia G. Effect of raloxifene and clodronate on bone density in postmenopausal osteoporotic women. Int J Tissue React. 2003;25(2):73-8.

5. Weinstein RS, Parfitt AM, Marcus R, Greenwald M, Crans G, Muchmore DB. Effects of raloxifene, hormone replacement therapy, and placebo on bone turnover in postmenopausal women. Osteoporos Int. 2003;14(10):814-22.

6. Heringa M. Review on raloxifene: profile of a selective estrogens receptor modulator. Int J Clin Pharmacol Ther. 2003;41(8):331-45.

7. Ahlert J. ICH Q8: Pharmaceutical Development. Regulatory Requirements Directed by the New Note for Guidance (EMEA/CHMP/167068/2004) in Comparison to the Previous Guideline (CPMP/QWP/155/96). A Critical View from the Generic Pharmaceutical Industry. Bonn: ICH; 2007. p. 34-40.

8. United States Pharmacopoeial Convention. USP XXXI. Métodos generales. The United States Pharmacopoeia Convention. 31 ed. Rockville: Mack Printing; 2008. p. 752-7.

9. Narang A, Rao V, Raghavan K. Excipient compatibility. In. Developing solid oral dosage forms, Pharmaceutical theory and practice. Cap. 6. ed. New York: Elservier Inc.; 2009. p. 125-45.

10. Fernández Cervera M, Pérez Sánchez I, Izquierdo Castro A, Jiménez Cuellar B, Yarie Camara F, Gustavo Fernández E. Technological development and stability study of meprobamate immediate released tablets. Rev Cubana Farm. 2010;44(4):456-64.

11. Waterman KC, Adami RC. Accelerated ageing: prediction of chemical stability of pharmaceuticals. Int J Pharm. 2005;293:101-25.

12. Steele G, Austin T. Preformulation Investigations using Small Amounts of Compound as an Aid to Candidate Drug Selection and Early Development. In Gibson M. (ed). Pharmaceutical Preformulation and Formulation. 2nd ed. New York: Informa Healthcare USA, Inc.; 2009. p. 17-129.

13. Regulación No 23. Requerimientos de los estudios de estabilidad para el registro de nuevos ingredientes farmacéuticos activos. Centro Estatal para el Control de Medicamentos (CECMED). La Habana, 2000. Disponible en: http://www.cecmed.sld.cu/Pages/Reg_EvalEL.htm

14. United States Pharmacopoeial Convention. USP XXXIII. The United States Pharmacopoeia Convention. 33 ed. Rockville: Mack Printing; 2010. p. 294, 4549.

15. Rodríguez Chanfrau JE, López Armas M. Validación de un método de análisis para la determinación de raloxifeno en una formulación cubana. Rev Cubana Farm. (en proceso de aparición).

16. ICH. Stability Testing: Photostability Testing of New Drug Substances and Products. ICH. Harmonised Tripartite Guideline Q1B. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Geneva: ICH; 1996.

17. Srinivas G, Kanumula G, Madhavan P, Kishore Kumar K, Rama Koti Reddy Y, Vishnu Priya M, et al. Development and validation of stability indicating method for the quantitative determination of Raloxifene hydrochloride and its related impurities using UPLC. J Chem Pharm Res. 2011;3(1):553-62.

18. Zhou D, Porter W, Zhang G. Drug Stability and Degradation Studies. In. Developing solid oral dosage forms, Pharmaceutical theory and practice. Cap. 5. New York: Elservier Inc.; 2009. p. 87-124.

19. Kontny MJ. Distribution of water in solid pharmaceutical systems. Drug Development and Industrial Pharmacy. 1988;14(14):1991-2027.

Recibido: 4 de marzo de 2013.
Aprobado: 6 de mayo de 2013.

Jorge Enrique Rodríguez Chanfrau. Centro de Investigación y Desarrollo de Medicamentos (CIDEM). Ave. 26 No. 1605 entre Boyeros y Puentes Grandes. CP 10600. Plaza de la Revolución. La Habana, Cuba. Correo electrónico: jorge.rodriguez@infomed.sld.cu