Role of Excipients on *N*-Oxide Raloxifene Generation from Raloxifene–Excipients Binary Mixtures

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Raloxifene HCl (RHCl) is known to be susceptible to oxidation and forms the corresponding *N*-oxide derivative as the primary degradation product. The purpose of this study was to evaluate the role of excipients on the generation of the *N*-oxide derivative from the corresponding RHCl–excipient binary mixtures. Binary mixtures of RHCl with crospovidone, povidone, magnesium stearate, Tween 80 and anhydrous lactose in drug: excipients ratio of 1 : 1 (crospovidone and povidone); 10 : 1 (Tween 80 and magnesium stearate) and 1 : 5 (anhydrous lactose) were prepared by both dry blending (trituration) and wet blending (to improve contact between drug and excipients). The prepared binary mixtures were then stored at 25, 40, 75 and 125 °C and generation of the *N*-oxide derivative was monitored over six months using a validated HPLC method. Pure drug and excipients stored similarly acted as controls. Further, all the individual excipients (used as control) were monitored for peroxide impurity generation using an in-house colorimetric method. The results showed that *N*-oxide generation was observed from all binary mixtures and the amount of *N*-oxide derivative formed were always higher from the mixtures prepared by wet blending and the amount of *N*-oxide derivative formed was dependent on storage temperature. This study thus shows that the presence of peroxide in the excipient and its role in oxidative degradation of drug substance calls for monitoring of the peroxide impurity present in the excipients used for formulating of drug sensitive to oxidation as used herein.

Key words raloxifene HCl; excipient; *N*-oxide-raloxifene; povidone; crospovidone

Incompatibility between drugs and excipients can alter the stability and bioavailability of drugs, thus affecting its safety and efficacy. Study of drug–excipient compatibility is an important and integral process in the development of a stable dosage form. Many reports are available in the literature that has deliberated the importance of this issue and various methods of compatability testing have been discussed.[1—5] Raloxifene HCl (RHCl), is a selective estrogen receptor modulator shown to be effective in the prevention of osteoporosis,[6] which could be a potential substitute for long-term female hormone replacement therapy. Raloxifene is available as 60 mg tablet under the brand of Evista (Eli-Lilly) and contains 12.5% w/w RHCl, 5% povidone, 6% crospovidone and a combination of other excipients including anhydrous lactose, lactose monohydrate, Tween 80 and magnesium stearate. In an earlier published report,[7] it was disclosed that raloxifene forms a *N*-oxide derivative as a degradation product when the tablets were stored in sealed bottles and open containers at both 30 °C/60% relative humidity (RH) and at 40 °C/75% RH. Stability studies on binary mixtures of RHCl with primary excipients used in the tablet (Evista) were reported,[7] wherein the binary mixtures were stored at 125 °C for a period of 31 d. The results showed that maximum amount of *N*-oxide derivative generation were observed in case of binary mixtures prepared with crospovidone and povidone and this was attributed to the presence of peroxide impurities in these excipients. The object of the present study is to conduct a long-term stability testing on raloxifene-excipient binary mixtures using a similar composition (to mimick the weight ratio that goes into tablet) as reported[7] and using different conditions of storage, so as to establish the role of excipients in the formation of the *N*-oxide derivative and also try to establish if a correlation exists between the amount of *N*-oxide formed and the level of peroxide impurity present in the excipients.

Experimental

Materials and Methods Raloxifene HCl and *N*-oxide raloxifene (Glochem Industries, Hyderabad, India), lactose (DMV International, Netherlands), Tween 80 (Qualigens, Mumbai, India) and magnesium sterate (Ferro, Portugal) were purchased from sources indicated. Povidone and crospovidone (International Speciality Products India Pvt. Ltd.) were in-house material. All other reagents used were of either analytical or HPLC grade, as appropriate.

Preparation of Binary Mixtures Binary mixtures of RHCl with each of the tablet core excipients as used in Evista[7] were prepared in mass ratios reflecting their approximate proportion in the formulation. On a w/w basis, the ratio of excipients to drug was 5 : 1 for anhydrous lactose, 1 : 1 for povidone and crospovidone, and 1 : 10 for magnesium stearate and polysorbate 80. The appropriate amount of each excipient was combined with 5.0 g of RHCl, and thoroughly mixed with mortar and pestle. The binary mixtures of similar compositions were also prepared by using appropriate amount of water into enough to wet the mixture. The prepared wet mixtures were dried in an oven, until the moisture content was equivalent to that of the corresponding dry binary mixtures, as determined by Sartorius moisture balance (MA 100H, Germany). The prepared mixtures were then placed in petri dish (75 mm diameter) and stored at 25, 40, 75 and 125 °C for six months.

HPLC Analysis of Degradant The *N*-oxide raloxifene generation in the binary mixtures was monitored using HPLC (Waters, U.S.A.) using 5-μm, 250×4.6 mm i.d. Inertsil C8 column (GL Sciences Inc., Japan) by a gradient elution method reported earlier.[7] The gradient elution utilized acetonitrile and a 75 mM phosphate buffer adjusted to pH 3.0 with 85% phosphoric acid. The initial mobile phase composition of 25% acetonitrile was maintained for 5 min, and then increased by 0.8%/min to final composition of 50% acetonitrile. RHCl degradants, including the *N*-oxide, were monitored using a UV detector at a wave length of 280 nm. *N*-Oxide derivative of raloxifene obtained from a commercial source was used as a standard.

Determination of Peroxide in Excipients Two grams of each excipient was added into 50 ml glass bottle, fitted with a cap and the weights were recorded. To this 48 ml of water was added and reweighed. All samples were prepared in triplicate. The bottles were placed on a shaker (Cole Palmer-Instrument company, U.S.A.), for 1 h (ideally to dissolve) and centrifuged, if undissolved, at 2500 rpm for 10 min, and the supernatant separated. One of the sample (clear solution or the supernatant, as the case maybe) was treated with 4 ml of 13% sulfuric acid and was considered as 'sample blank' and the remaining two were treated with 4 ml of titanium(III) chloride–sulfuric acid reagent. The treated samples were capped, mixed thoroughly and kept for 30 min at ambient temperature (25 °C). The samples were measured at 405 nm using a spectrophotometer (Agilent 8453, China) against water as blank.

Results and Discussion

Binary drug–excipient mixtures were evaluated to identify contributors to the formulation of *N*-oxide derivative of

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Table 1. Raloxifene *N*-Oxide Levels for Binary Mixtures from Day 0 to Day 180 (*N*-Oxide Expressed as % w/w of Raloxifene)

Drug–excipient mixture (ratio)	Mean degradant amount												
	Day 0	Day 30				Day 90				Day 180			
		25 °C	40 °C	75 °C	125 °C	25 °C	40 °C	75 °C	125 °C	25 °C	40 °C	75 °C	125 °C
Crospovidone : RHCl (1 : 1)	ND	ND	ND	ND	0.10± 0.0071	0.02± 0.0014	0.02± 0.0021	0.06± 0.0071	0.12± 0.0141	0.02± 0.0014	0.03± 0.0021	0.05± 0.0007	0.13± 0.0021
Anhydrous lactose : RHCl (5 : 1)	ND	ND	ND	ND	0.01± 0.0014	ND	ND	0.04± 0.0014	0.06± 0.0021	0.01± 0.0014	0.03± 0.0028	0.05± 0.0014	0.07± 0.0014
Povidone : RHCl (1 : 1)	ND	ND	ND	ND	0.12± 0.0141	0.01± 0.0007	0.02± 0.0014	0.04± 0.0028	0.11± 0.0014	0.03± 0.0021	0.06± 0.0014	0.05± 0.0021	0.14± 0.0071
Magnesium stearate : RHCl (1 : 10)	ND	ND	ND	ND	0.05± 0.0021	0.03± 0.0014	0.01± 0.0014	0.02± 0.0028	0.02± 0.0014	0.03± 0.0007	0.07± 0.0014	0.05± 0.0007	0.06± 0.0014
Tween : RHCl (1 : 10)	ND	ND	ND	ND	ND	0.02± 0.0028	0.02± 0.0014	0.05± 0.0021	0.07± 0.0021	0.06± 0.0028	0.07± 0.0014	0.07± 0.0028	0.09± 0.0071
Crospovidone : RHCl (1 : 1) granulated with water (sprinkled)	ND	0.02± 0.0021	0.05± 0.0021	0.15± 0.0071	0.30± 0.0141	0.03± 0.0014	0.08± 0.0021	0.13± 0.0071	0.32± 0.0141	0.03± 0.0028	0.13± 0.0021	0.13± 0.0141	0.52± 0.0071
Anhydrous lactose : RHCl (5 : 1) granulated with water (sprinkled)	ND	ND	ND	ND	0.04± 0.0014	ND	0.04± 0.0014	0.04± 0.0014	0.07± 0.0021	0.04± 0.0007	0.04± 0.0071	0.06± 0.0021	0.09± 0.0014
Povidone : RHCl (1 : 1) granulated with water (sprinkled)	ND	ND	0.03± 0.0014	0.05± 0.0028	0.15± 0.0212	0.02± 0.0007	0.09± 0.0021	0.12± 0.0141	0.22± 0.0071	0.02± 0.0007	0.10± 0.0212	0.13± 0.0141	0.03± 0.0021
Magnesium stearate : RHCl (1 : 10) granulated with water (sprinkled)	ND	ND	ND	0.02± 0.0021	0.04± 0.0014	ND	0.02± 0.0014	0.03± 0.0028	0.04± 0.0014	0.03± 0.0014	0.06± 0.0021	0.05± 0.0021	0.08± 0.0014
Tween : RHCl (1 : 10) granulated with water (sprinkled)	ND	ND	ND	0.04± 0.0014	0.04± 0.0021	ND	0.04± 0.0021	0.05± 0.0021	0.08± 0.0071	0.01± 0.0028	0.04± 0.0014	0.07± 0.0071	0.11± 0.0141

ND: not detected.

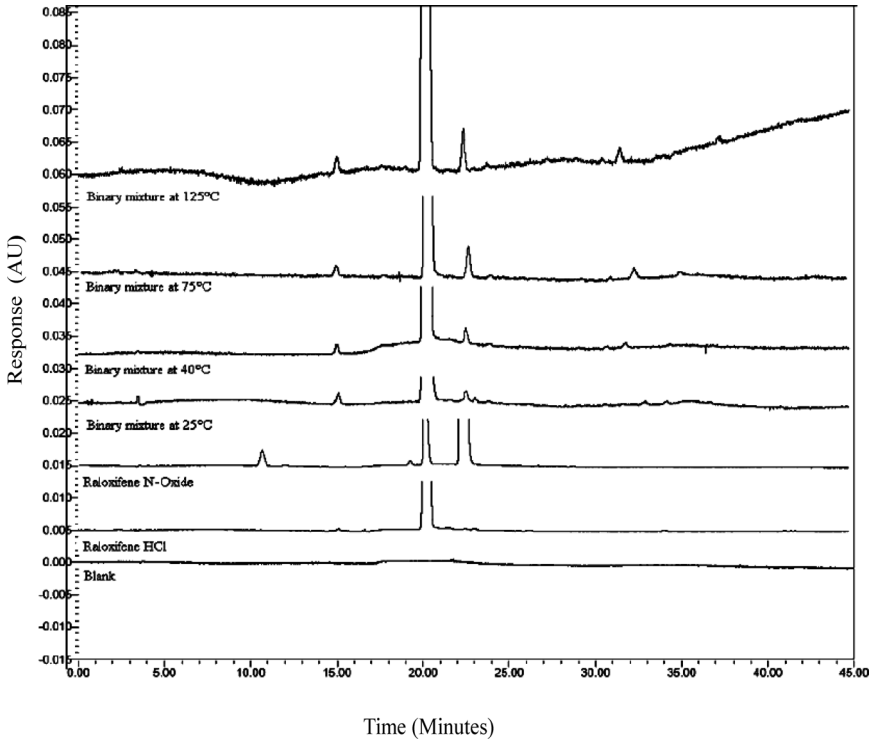

Fig. 1. HPLC Chromatogram of *N*-Oxide, Stressed Binary Mixtures

raloxifene for a relatively longer period of time and at different storage temperatures than that reported earlier. The amount of *N*-oxide degradant formed with each of the excipients after 30, 90 and 180 d of storage at the different temperatures are shown in Table 1.

The results show that the amount of *N*-oxide degradant formed was higher for binary mixture prepared by wet blending than the corresponding mixtures prepared as a dry blend. The prepared wet binary mixtures were dried until the point where the moisture content of these mixtures were equivalent to the corresponding mixtures prepared as dried blend. It was done in order to rule out the possible effect of additional moisture on possible degradation of the drug. Usage of water for preparing the blend could have enhanced the proximity of the drug to the excipient to facilitate for the solid-state reaction to occur. In general, the amount of *N*-oxide derivative formed was dependent on storage of temperature, being higher when stored at 125 °C.

Also the *N*-oxide derivative generation was observed from all the binary mixtures and the amount of *N*-oxide derivative formed were always higher from the mixtures prepared by wet blending and the amount of *N*-oxide derivative formed was dependent on storage temperature. Maximum amount of *N*-oxide was formed from binary mixture of crospovidone and povidone, followed by magnesium stearate, which was quite surprising, since the drug to magnesium stearate % ratio was approximately 90 to 10 as opposed to % ratio of 50 to 50 for binary mixtures with crospovidone and povidone.

The amount of peroxide impurities determined by in-house UV–Visible spectroscopy method is shown in Table 2. The Table shows that out of the excipients studied povidone and crospovidone contain high levels of peroxide followed by Tween, lactose and magnesium stearate. Unlike the previous report,[7] wherein substantial amount of *N*-oxide derivative

Table 2. Peroxide (in ppm) Levels in Excipients

Crospovidone

Period in days	125 °C	75 °C	40 °C	25 °C
0	45±2.8	45±2.8	45±2.8	45±2.8
30	125±4.2	97±3.5	80±2.8	77±1.4
90	140±2.1	108±4.2	99±3.5	81±3.5
180	220±4.2	181±9.1	175±4.2	s135±4.9

Povidone

Period in days	125 °C	75 °C	40 °C	25 °C
0	27±2.1	27±2.1	27±2.1	27±2.1
30	55±3.5	41±1.4	36±3.5	34±2.8
90	92±1.4	77±2.8	68±2.1	60±1.4
180	141±4.9	130±2.1	121±3.5	117±2.8

Magnesium stearate

Period in days	125 °C	75 °C	40 °C	25 °C
0	10±2.8	10±2.8	10±2.8	10±2.8
30	19±2.1	17±1.4	16±1.4	15±2.1
90	22±1.4	21±2.1	20±2.1	18±2.1
180	24±2.8	23±1.4	20±2.8	19±2.1

Tween 80

Period in days	125 °C	75 °C	40 °C	25 °C
0	24±2.8	24±2.8	24±2.8	24±2.8
30	32±1.4	28±2.8	27±2.1	26±2.1
90	40±2.8	37±2.1	35±4.2	34±2.1
180	51±2.1	50±2.8	50±3.5	48±1.4

Anhydrous lactose

Period in days	125 °C	75 °C	40 °C	25 °C
0	12±1.4	12±1.4	12±1.4	12±1.4
30	21±2.8	18±2.1	17±2.1	14±1.4
90	22±2.1	19±2.8	17±1.4	17±1.4
180	25±1.4	21±2.1	19±2.8	20±2.1

was observed only for binary mixtures raloxifene containing povidone and crospovidone, in our study we observed that appreciable amount of *N*-oxide derivative generation even with binary mixtures containing Tween 80, magnesium stearate and lactose.

It has been reported that Tween 80 contains appreciable amounts of hydroperoxide in the range of 1100 to 3900 nmol/g depending the source of Tween 80 while magnesium stearate and lactose usually contain <100 nmol/g of peroxides by a more sensitive ferrous oxidation-xylenol orange (FeOX2) method reported recently.[8)]

The degradation of raloxifene HCl is an example of two electron nucleophilic reaction that can occur between the peroxide and the drug substance. Low levels of peroxide content, as reported for magnesium stearate and lactose, may also be sufficient to participate is peroxy radical chain propagation, which could have been initiated at higher temperatures as used in the study. The radicals could behave as initiators for radical chain processes leading to significant peroxide and radical concentrations from a small amount of starting peroxide. These radical processes could be responsible for the formation of *N*-oxide derivatives from binary mixtures contain Tween 80, lactose and magnesium stearate, when stored for prolonged periods of time at temperature of 125 °C, which was otherwise unobserved in the study reported earlier.

Conclusion

Our study shows that raloxifene is capable of oxidatively degraded to its *N*-oxide derivative by other excipients like Tween 80, lactose and magnesium stearate which usually contains considerably lesser amount of peroxide than povidone and crospovidone though the amount of *N*-oxide derivative formed is less than 0.2%. Hence the role of peroxide in the excipient, however less they may be, in the oxidative degradation of a drug substance necessitates monitoring the peroxide content of excipients and formulated product, especially if a drug sensitive to oxidation is used as in the present study. Even when oxidation of drug substance is not a problem, the peroxide level in the excipients may also lead to other formaultations challenges like for example change in pH, appearance and viscosity and the decomposition of peroxide may yield to unwanted impurities like aldehydes and carboxylic acids. In our opinion, acceptable peroxide limits can only be assigned within the content of a particular formulation that is known to be sensitive to peroxides and oxidation and by understanding the relationship between peroxide and formulation quality.

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