Research Article

FORCED DEGRADATION STUDY OF STRONTIUM RANELATE (ANTI-OSTEOPORETIC DRUG)

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ABSTRACT

Strontium ranelate (SR) is chemically designated as distrontium 5-[bis(2-oxido-2-oxoethyl)amino]-4-cyano-3-(2-oxido-2-oxoethyl)thiophene-2-carboxylate is used for treating osteoporosis as well as postmenopausal osteoporosis. SR has a dual mode of action, both increasing bone formation and decreasing bone resorption, which rebalances bone turnover in favour of bone formation and increases bone strength. Forced degradation studies help facilitate pharmaceutical development as well in areas such as formulation development, manufacturing, and packaging, in which knowledge of chemical behaviour can be used to improve a drug product. ICH guidelines (Q1A(R2) and Q1B) requires that stress testing should be carried out to elucidate the substance. The present study involved the effect of acid, alkali, light, hydrogen peroxide and temperature on the stability of SR. HPLC method has been developed to study the SR in pure form as well as for degradation products. In the present work, degradation study using HPLC according to ICH guides for strontium ranelate show that thermal treatment, UV light (upto 24 hrs) and alkali treatment have negligible effect on the degradation of SR. Whereas acidic and oxidative environment degrade the API to significant extent.

Keywords: Osteoporosis, Strontium ranelate, Forced degradation, ICH guidelines.

INTRODUCTION

Strontium ranelate (SR) is chemically designated as distrontium 5-[bis (2-oxido-2- oxoethyl) amino]-4-cyano-3-(2-oxido-2-oxoethyl) thiophene-2-carboxylate is used for the treatment of osteoporosis[1-3] as well as postmenopausal osteoporosis (figure 1).

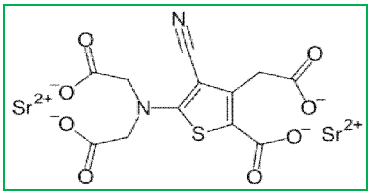

Figure 1: Chemical structure of strontium ranelate

The objective of treatment is to either follow anti-resorptive or bone forming strategies[3]. Currently available medications, such as bisphosphonates, selective estrogen receptor modulators, and teriparatides, have shown their ability to reduce vertebral and/or nonvertebral fractures[1]. But it remains sub-optimal. There is, therefore, an urgent need of new effective, safe, and user-friendly medications to optimize the treatment of postmenopausal osteoporosis. Among these recent advances SR has gained importance in the treatment and prevention of osteoporosis[4]. SR, a novel orally active agent consisting of two atoms of stable strontium and the organic moiety ranelic acid, has been developed for the treatment of osteoporosis[5,6]. SR is a new antiosteoporotic treatment with a dual mode of action, both increasing bone formation and decreasing bone resorption, which rebalances bone turnover in favour of bone formation and increases bone strength. It has been shown to enhance osteoblastic cell replication and increase collagen synthesis while it decreases preosteoclast differentiation and bone-resorbing activity of mature osteoclasts *in vitro*[1]. The antifracture efficacy of strontium ranelate, 2 g per day orally, in the treatment of postmenopausal osteoporosis has been investigated in a large-scale, international, multicenter, phase 3 programme with more than 7000 patients[7]. A significant early (after 1 year) and sustained (over 3 years) antifracture efficacy of strontium ranelate, compared with placebo, was demonstrated in patients with prevalent vertebral fracture with reductions in risk of new vertebral fracture of 49% after 1 year ($P < 0.001$) and 41% over 3 years ($P < 0.001$). In addition, the relative risk of clinical vertebral fracture was significantly reduced by 52% ($P = 0.003$) after 1 year and by 38% ($P < 0.001$) over 3 years in the strontium ranelate group compared with placebo.

Literature survey reveals that high-performance liquid chromatography (HPLC) and reversed phase (RP)-HPLC for determination of content uniformity and simultaneous estimation of SR is reported[8], but there is no degradation study and related RP-HPLC method available in open literature for the determination of strontium ranelate as an API. The main objective of this study was, therefore, (i) to carry out degradation study to study the effect of acid, alkali, light, hydrogen peroxide and temperature (ii) to develop a new, simple, economical, selective, precise and reproducible RP-HPLC method which can detect the degradation products formed even in very small amounts using ultra-violet (UV) detection.

Forced degradation studies may help to facilitate pharmaceutical development as well in areas such as formulation development, manufacturing, and packaging, in which knowledge of chemical behaviour can be used to improve a drug product. The available regulatory guidance provides useful definitions and general

comments about degradation studies[7]. The International Conference on Harmonization (ICH) guidelines[11,12] indicates that stress testing is designed to determine the intrinsic stability of the molecule by establishing degradation pathway in order to identify the likely degradation products and to validate the stability indicating power of the analytical procedure used. ICH guidelines 'stability testing of new drug substances and products' Q1A(R2)[11] and (Q1B)[12] requires that stress testing should be carried out to elucidate the substance. It suggests that the degradation products that are formed under the variety of condition should include the effect of temperature, appropriate oxidation, photolysis and susceptibility to hydrolysis across a wide range of pH value. In the guideline, the study of effect of temperature is suggested to be done in 10°C increment above the accelerated temperature (50°C, 60°C etc.) and that of humidity at a level of 75 % or greater. No exact details are however provided for the study of oxidation, photolysis and hydrolysis at different pH values[10].

MATERIALS AND METHODS

Materials

Strontium ranelate was obtained as a gift sample from Enaltech lab Pvt. Ltd., Mumbai, India. High purity water was prepared by using Millipore Milli-Q plus water purification system. Other chemicals used in this study were Methanol, Potassium dihydrogen orthophosphate buffer, Orthophosphoric acid, hydrochloric acid, sodium hydroxide and hydrogen peroxide (all LR Grade).

Instrument used

The instruments used were HPLC, pH meter, Electronic analytical balance, centrifuge, UV chamber and Sonicator. The HPLC system details are as follows: waters alliance RP- HPLC with 2487 UV detector and Empower software, was used for all the experiments. The column used was Inertsil Luna C18 column (250 x 4.6 mm, 5 µ particle diameter).

Methodology

Chromatographic conditions

Chromatographic separation was achieved at ambient temperature (25°C) on a reversed phase column using a mobile phase consisting of a mixture of Methanol: Buffer solution (50 mM potassium dihydrogen orthophosphate at pH 3.0) in the ratio (1:3). The mobile phase so prepared was filtered through 0.45 µm membrane filter and degassed by sonication. Flow rate of 0.8 ml/min was maintained. The injection volume was 20 µL for all the analysis. The detection was carried out at wavelength of 323 nm.

Preparation of buffer

Dissolve 6.8 g (50 mM) of potassium dihydrogen phosphate in 950 ml distilled water. Adjust pH of solution to 3.0 with orthophosphoric acid. Make the volume to 1000 ml.

Standard preparation

100 mg of strontium ranelate working standard was accurately weighed and transferred to a 100 ml volumetric flask. Solution was sonicated and diluted up to the mark with buffer solution.

Sample preparation

Injection sample equivalent to 50 mg of strontium ranelate was transferred to a 100 mL volumetric flask. About 60 mL of buffer solution was added and the solution was sonicated for 15 min and make up to the mark with buffer solution. The resulting solution was filtered through 0.45 µm membrane filter. The solution was mixed well and centrifuged at 2500 rpm for 10 min.

FORCED DEGRADATION STUDIES

Figure 2 shows the flow chart of forced degradation studies according to ICH guidelines carried out in present wok. The hydrolytic degradation of a new drug in acidic and alkaline condition can be studied by refluxing the drug in acid or base with some strength (such as 0.1N, 1N etc). If reasonable degradation is seen, testing can be stopped at this point. However in case no degradation is seen under these conditions the drug should be refluxed in acid/alkali of higher strength and for longer duration of time. Alternatively if total degradation is seen after subjecting the drugs to initial condition, acid/alkali strength can be decreased with decrease in reaction temperature. To test for oxidation, it is suggested to use hydrogen peroxide in the concentration range of 3 to 30 %. In some drugs extensive degradation is seen when exposed to 3% of hydrogen peroxide for very shorter time period at room temperature. The drug solution should be exposed to UV radiation, in UV chamber for appropriate time to study the photolytic stability of the drug.

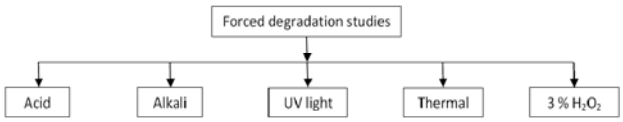

Figure 2: Forced degradation studies flow chart[10]

Another practical aspect of stress testing that generates enquiries from practitioners is one that concern the best way to handle samples containing high concentrations of acid, alkali or oxidizing agent for HPLC[13]. One approach is to dilute the sample enough so that the concentration of reagent falls within the acceptable range. For HPLC the dilution can be performed in the mobile phase, where as for TLC a suitable solvent such as methanol or ethanol can be used. The second approach involves neutralization of acid and alkali solutions to tolerable pH. Dilution is often easier than neutralization. The problems with neutralisations are that it is difficult to perform in a quantitative manner and moreover it generally leads to precipitation of the dissolved ingredients of the sample. That can be controlled by filtration of that sample by syringe filter. In the present work, dilution with mobile phase has been carried out.

Preparation of acid and base induced degradation Product

Injection sample equivalent to 100 mg of strontium ranelate was transferred to 100 mL volumetric flask. To it, 10 ml of mobile phase was added and sonicated for 15 min with intermittent shaking. To it 5 ml of 1 N HCl was added and 5 ml of 1N NaOH were added separately. The sample was heated on a boiling water bath for 30 min, cooled to room temperature and diluted to volume with mobile phase, mixed well. The acidic forced degradation and the alkaline forced degradation was performed in dark in order to exclude the possible degradative effect of light. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC system.

Preparation of hydrogen peroxide – induced degradation product

Injection sample equivalent to 100 mg of strontium ranelate was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking. To it 5 mL of 3.0% H_2O_2 was added. The sample was heated on a boiling water bath for 30 min, cooled to room temperature and diluted to volume with mobile phase, mixed well. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC system.

Preparation of photo-degradation

Injection sample equivalent to 100 mg of strontium ranelate (previously kept in UV light for 24 hr) was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking and diluted up to the mark with mobile phase. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC system.

Preparation of thermal degradation product

Injection sample equivalent to 100 mg of strontium ranelate was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking. The sample was heated on a boiling water bath for 30 min, cooled to room temperature and diluted to volume with mobile phase, mixed well. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC**.** The no stress treatment sample (as control) has been evaluated relative to the standard concentration where as rest of the stressed condition samples are evaluated relative to the control sample with respect to the % assay and % degradation. The percentage degradation results are calculated by area normalization method.

RESULTS AND DISCUSSION

Method validation

Linearity

The linearity of response for strontium ranelate assay method was determined by preparing and injecting solutions with concentrations of about 50, 100, 150, 200 and 250 ppm of strontium ranelate. The linearity of peak area responses verses concentration was studied and a calibration curve was plotted. It shows that strontium ranelate have linearity in the range of 50-250 ppm. The results have been shown in table 1 and figure 3.

Table 1: Linearity study for strontium ranelate

S.No	Statistical Parameters	HPLC
1	Concentration range	50-250 ppm
2	Regression Equation	Y = 33472x + 5000
3	Correlation Co-efficient	0.999
4	Slope	33472
5	Intercept	5000

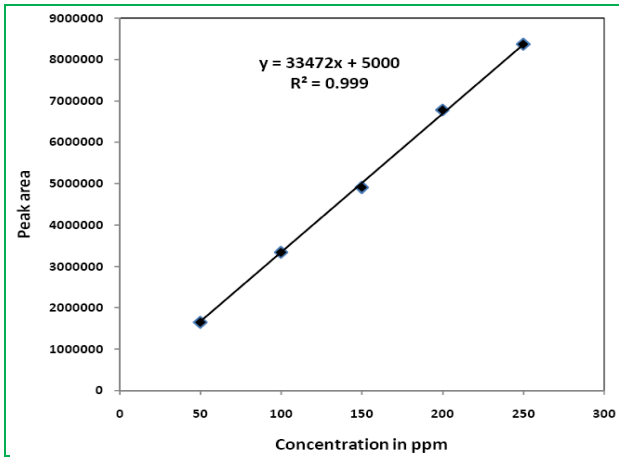

Figure 3: Linearity study

Robustness study

Two different columns were tried as under same chromatographic conditions namely, RP18, 250 x 4.6 mm, 5 µ and Luna C8 (Octylsilane), 250 x 4.6 mm, 5 µ. Luna C8 gave good peak shape but a lower retention with low peak purity. RP18 column had given a good peak shape with response at affordable retention time with peak purity of strontium ranelate on higher side. The results are reported in table 2.

Precision

Precision was measured in terms of repeatability of application and measurement. Repeatability was carried out using six replicates of the same standard concentration (200 µg / mL for standard application). For precision study, three samples from same preparation and three different preparation have been used. Precision studies were carried out by estimating the corresponding responses. The % RSD values have been shown in below table 3.

Table 2: Robustness study for strontium ranelate

Sr. No.	Concentration	Column	Day	Analyst	RT (min)	Peak area	% Assay
1	200 ppm	C18	22 Aug 11	A	7.421	6787219	101.31
2	200 ppm	C18	22 Aug 11	B	7.605	6681719	99.74
3	200 ppm	C8	8 Sept 11	A	6.644	6148706	91.78

Table 5: Stressed study data of Strontium ranelate

Condition	% Assay	RT of drug (min)	RT of impurities (min)	% Degradation
No stress treatment	99. 27	7.42		nil
Acid	77.15	7.05	8.30,12.11, 19.15,	20.93
Alkali	97.59	6.94	8.20	1.86
H_2O_2	80.89	7.38	8.62,12.76,20.55,29.15	18.59
thermal	94.77	7.30	8.55,12.75,29.98	4.46
UV light	98.19	7.17	12.37	0.71

Table 6: Summary of forced degradation study

Stress condition	Time	% Assay of active substance	Mass balance (% Assay of drug + % Degradation products)
Acid degradation (1 N HCl)	1/2 hr	77.15	77.15 + 22.85 = 98.04
Alkali degradation (1N NaOH)	1/2 hr	97.59	97.59 + 1.86 = 99.45
H_2O_2 degradation (3%)	1/2 hr	80.89	80.89 + 18.59 = 99.48
Thermal degradation (60°C)	1/2 hr	94.77	94.77 + 4.46 = 99.23
UV degradation	24 hr	98.19	98.19+0.71 =98.90

Table 3: Method precision for strontium ranelate

Sample	% Assay	% Deviation from mean assay value
1	99.86	0.59
2	99.97	0.70
3	98.66	-0.61
4	97.98	-1.29
5	98.55	-0.72
6	100.6	1.33
Mean	**99.27**	
± SD	**1.02**	
% RSD	**1.02**	

Accuracy

Table 4 shows the accuracy study of strontium ranelate.

Table 4: Accuracy study

% Level	Peak area	% Assay value
50	4913559	97.76
100	6787219	101.31
150	8377736	100.05

Degradation study results

The chromatogram of no stress treatment sample (as standard) showed no additional peak (figure 4). The retention time (RT) of standard and sample were 7.4 min. The chromatogram of acid degraded sample (figure 5) showed seven additional peaks. The major degradation products were obtained at retention time of 8.3 min (% area = 1.27), 12.1 min (% area = 12.22) and 19.1 min (% area = 7.44). The chromatogram of alkali degraded sample (figure 6) showed only one additional peak with significant % area (1.86) at 8.2 min. The chromatogram of hydrogen peroxide degraded sample (figure 7) showed four significant additional peaks at RT of 8.6 min (% area = 2.22), 12.7 min (% area = 6.00), 20.5 min (% area = 1.09) and 29.5 min (% area = 9.28). The chromatogram of thermal degraded sample (figure 8) showed only one degradation peak with significant contribution at RT of 12.7 min (% area 1.25). The chromatogram of UV degraded sample (figure 9) showed one significant peak at RT of 12.3 min with % area of 0.71.

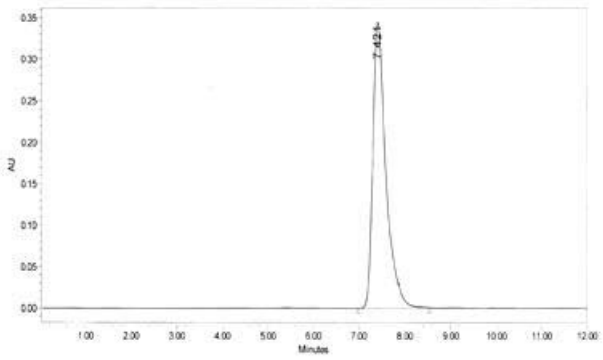

Figure 4: Chromatogram of working standard of strontium ranelate

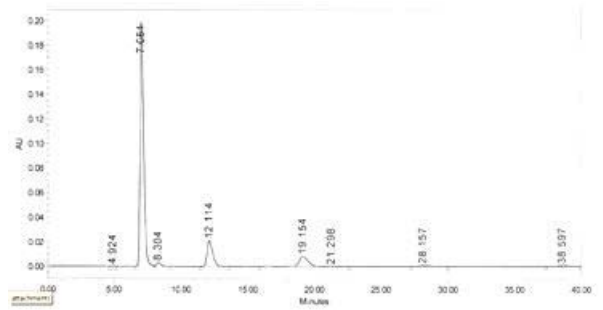

Figure 5: Chromatogram of sample from acid degradation study

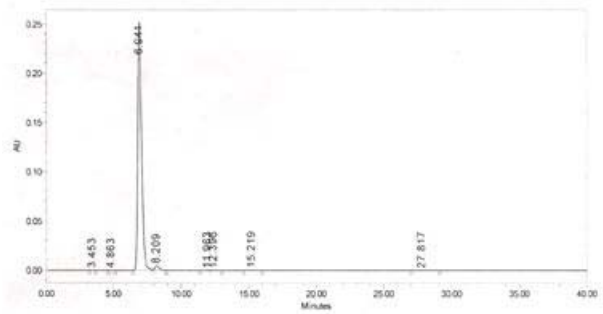

Figure 6: Chromatogram of sample from alkali degradation study

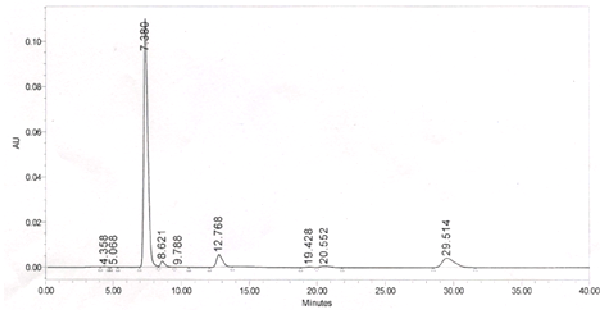

Figure 7: Chromatogram of sample from hydrogen peroxide degradation study

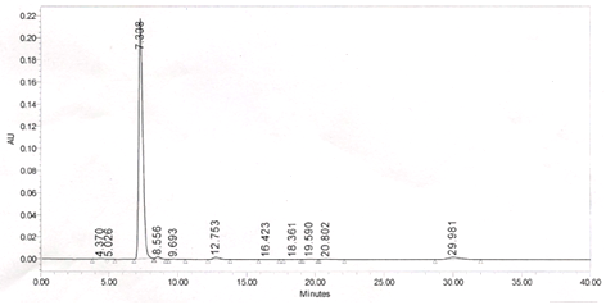

Figure 8: Chromatogram of sample from thermal degradation study

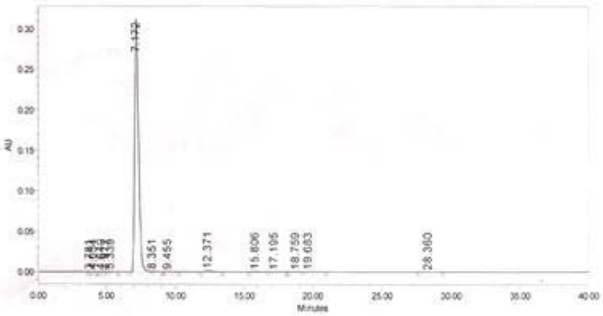

Figure 9: Chromatogram of sample from photo-degradation study

CONCLUSION

It can be concluded that the effect of temperature, UV light (up to 24 hours) and alkali treatment was considerably lower compared to acid and hydrogen peroxide. In each forced degradation study of strontium ranelate, additional peaks were observed. The response of the drug was changing from the initial control sample the values were depicted in table 5 and 6. This indicates that the drug is not susceptible to base hydrolysis degradation, UV degradation (up to 24 hrs) and thermal degradation but susceptible to acid hydrolysis and to hydrogen peroxide oxidation.

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