ISSN 0975-6299

Vol 2/Issue 2/Apr-Jun 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

ANALYTICAL CHEMISTRY

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF STRONTIUM RANELATE IN SACHET

K.MYTHILI*, S.GAYATRI, KOYA RAVI TEJA, K.CHITRA AND C.UMA MAHESWARA REDDY

Faculty of Pharmacy, Sri Ramachandra University, Porur, Chennai, Tamil Nadu, India

K.MYTHILI

Faculty of Pharmacy, Sri Ramachandra University, Porur, Chennai, Tamil Nadu, India

ABSTRACT

A rapid and sensitive RP-HPLC method with UV detection (323 nm) for routine analysis of strontium ranelate in bulk and in pharmaceutical formulation as sachet was developed. Chromatography was performed with mobile phase containing a mixture of monobasic potassium phosphate buffer and methanol (3:1 v/v) with the flow rate of 0.8 ml/min at 25 º C. In the range of 50-250 µg/ml, the linearity of strontium ranelate shows a correlation coefficient of 0.999. The percentage recovery studies were found to be in the range of 97-102 %. The proposed method was validated by determining accuracy, precision and ruggedness. The results of all the validation parameters were well within their acceptance values.

ISSN 0975-6299 Vol 2/Issue 2/Apr-Jun 2011

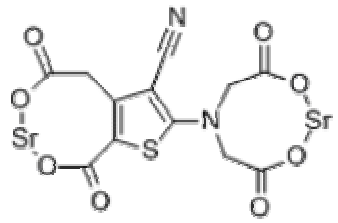
KEY WORDS

Strontium ranelate, RP- HPLC, UV detection, Validation

INTRODUCTION

Strontium ranelate, a strontium (II) salt of ranelic acid, is a medication for osteoporosis[1-2]. Chemically strontium ranelate is distrontium 5-[bis (2- oxido-2-oxo ethyl) amino]-4-cyano-3-(2-oxo ethyl) thiophene-2-carboxylate[3-5]. The literature survey showed that RP-HPLC method was developed for the determination of strontium ranelate and its related substances by using the mobile phase containing a mixture of tetrabutylammonium hydroxide solution and methanol[6]. The present research work was aimed to estimate and validate a simple, specific, accurate and rapid RP-HPLC method for the estimation of strontium ranelate in pure and its sachet formulation by using the mobile phase containing a mixture of monobasic potassium phosphate buffer and methanol.

Structure of strontium ranelate



MATERIALS AND METHODS

Chemicals and Reagents :
Strontium ranelate was obtained as a gift sample from Sai Adventium Pharma Ltd., Hyderabad, Andhra Pradesh, India. Water used was of HPLC grade and all other chemicals of analytical grade were used.

Instruments :
The instruments used were HPLC (Shimadzu), pH meter (Systronics), Electronic analytical balance (Shimadzu), and Sonicator (Toshniwal).

Preparation of buffer :
6.8 g of potassium phosphate mono basic was weighed accurately and transferred into a beaker containing 950 ml of Milli-Q water. The material was dissolved well. The pH was adjusted to 3.0 with phosphoric acid. It was transferred to 1000 ml volumetric flask and volume was made up to the mark with water. It was filtered through 0.45 µ filter.

Preparation of mobile phase :
The mobile phase was prepared by mixing 750 ml of buffer with 250 ml of methanol. Then the solution was sonicated for 15 min and filtered through 0.45 µ membrane filter paper.

Preparation of standard stock solution of strontium ranelate :
50 mg of Strontium ranelate was weighed accurately and transferred into a clean 100 ml volumetric flask and made up to the volume with buffer solution.

Preparation of working standard solution :
From the stock solution 10, 20, 30, 40 and 50 ml were pipetted into 5 different 100 ml volumetric flask and the volume was made up to the mark with buffer so as to get the concentration ranging from 50-250 µg/ml.

Assay of strontium ranelate in sachet :

The contents of five sachets were mixed and weighed. The average weight of each sachet was found out. A quantity of powder equivalent to 50 mg of strontium ranelate was weighed accurately and transferred into a clean 100 ml volumetric flask and dissolved in buffer solution. Then the solution was made up to the volume with buffer. The solution was sonicated for 15 min and filtered through 0.45 µm membrane filter. Then the solution was further diluted with buffer to obtain the concentrations of 100 µg/ml, 150 µg/ml and 200 µg/ml.

The above working standard and sample solutions were injected in to the column at the intervals of 10 minutes.

Chromatographic conditions :

The HPLC system consisted of an isocratic pump, an auto sampler and a UV detector. The stationary phase used was Phenomenex C_{18} column (250×4.6 mm), 5 µ particle size and the mobile phase used was monobasic potassium phosphate buffer (pH adjusted to 3): methanol (3:1 v/v). Chromatography was performed at 25 º C at a flow rate of 0.8 ml/min and the detection was performed by an UV spectrophotometer at the wavelength of 323 nm. The injection volume was 20 µl. The run time was set to 10 minutes.

Peak area of the prepared standard and sample solutions were obtained by using the above mentioned chromatographic conditions. The amount[7] of strontium ranelate was calculated by using formula

$$\text{Amount} = \frac{\text{Test peak area} \times \text{Standard dilution}}{\text{Standard peak area} \times \text{Test dilution}}$$

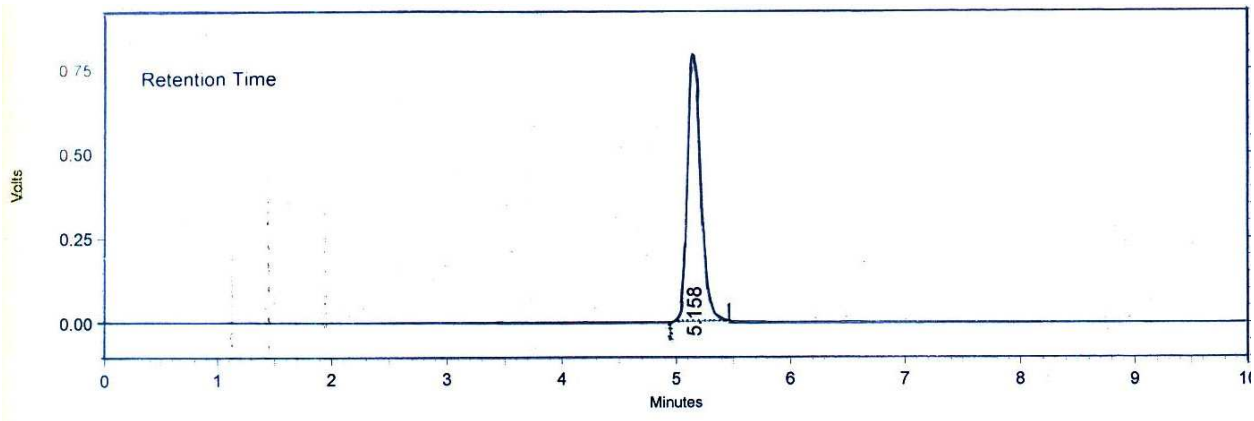
Method Validation :

The proposed method was validated for the parameters like linearity, accuracy, precision and ruggedness[8-9]. In addition system suitability parameters[10] were also calculated.

RESULTS AND DISCUSSION

The best peak shape and maximum separation was achieved with the mobile phase composition comprising a mixture of monobasic potassium phosphate buffer and methanol (3:1 v/v). The retention time of 5.1 min enable rapid determination of the drug, which is important for routine quality control analysis.

Figure-1
Chromatogram



System suitability Tests :

The system suitability test was performed to ensure that the complete testing system was suitable for the intended application. System suitability was daily performed during the entire validation of this method. The results were showed in Table 1.

Table - 1
System Suitability Tests

S.No	Parameters	Results
1	Retention time	5.1 min
2	Theoretical plates	9221
3	Asymmetry	1.2

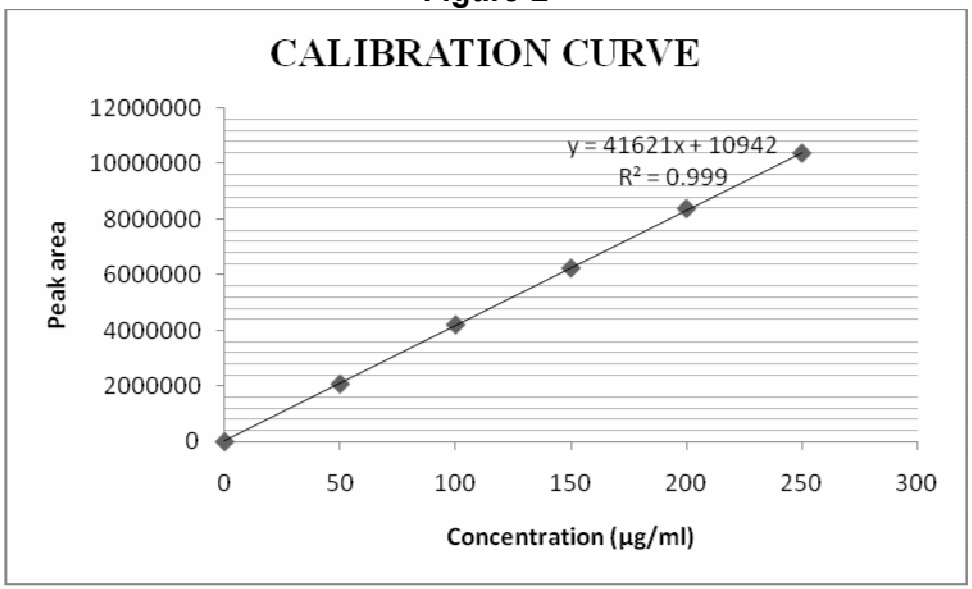
Linearity :

The linearity of peak area responses versus concentration was studied from 50-250 µg/ml for strontium ranelate. A linear response was observed over the examined concentration and calibration curve was plotted. The results were shown in Table-2 and Figure-2.

Table - 2

S.No	Statistical Parameters	HPLC
1	Concentration range	50-250 µg/ml
2	Regression Equation	y = 41621 x + 10942
3	Correlation Co-efficient	0.999
4	Slope	41621
5	Intercept	10942

Figure-2



Accuracy :

Accuracy of this method was evaluated by carrying out the recovery studies. Sample solution was spiked with strontium ranelate standard solution. The results were shown in Table-3.

Table – 3

S.No	% LEVEL	PEAK AREA	ASSAY VALUE (%)	% RECOVERY
1	50	6428553	103.00	97.89
2	100	8668298	104.16	102.77
3	150	10138444	97.46	98.07

Precision :

The intraday and inter day precision studies ware carried out by estimating the corresponding responses of the analysis on the same day and on 3 different days. The % RSD values were shown in Table-4.

Table - 4

Precision parameter		Assay value (%)	% RSD
Intraday precision	Initial	99.99	0.1061
	After 3 hrs	99.81	0.021
	After 6 hrs	99.71	0.092
Inter-day precision	Day 1	99.96	0.0353
	Day 2	99.95	0.0283
	Day 3	99.83	0.0566

Repeatability

The method passed the test for repeatability as determined by percentage RSD of the peak area of 6 replication injection at 100 percent test concentration. The results were shown in Table-5.

Table – 5

S.No	PEAK AREA	ASSAY VALUE (%)	%RSD
1	4206619	101.09	0.011
2	4215731	101.31	0.108
3	4197935	100.88	0.082
4	4210041	101.17	0.046
5	4199794	100.93	0.065
6	4203351	101.01	0.024

Ruggedness

Ruggedness was determined by carrying out the experiment by different analyst on different days. The results were shown in Table-6.

Table – 6

S.No	PEAK AREA	ASSAY VALUE	% DEVIATION
Analyst-1	6231542	99.83	0.17
Analyst-2	6225653	99.73	0.26
Day-1	6207932	99.45	0.54
Day-2	6199543	99.32	0.68

CONCLUSION

The RP-HPLC method with UV detection for the assay of strontium ranelate was developed and validated. The proposed HPLC method enables a fast quantitative determination of strontium ranelate in sachet formulation. The results showed that the method is very selective, accurate, reproducible and sensitive. No significant interfering peak was detected in chromatogram. As the run time is only 10 min, this method is very economical in regular use. The method involves use of a simple mobile phase and minimum sample preparation, encouraging its application in quality control for the analysis of strontium ranelate in raw material and sachet formulation.

ACKNOWLEDGEMENT

The authors are thankful to Adventium Pharma Ltd., Hyderabad for providing the gift sample of strontium ranelate and also thankful to the management of Sri Ramachandra University for their encouragement and providing the necessary facilities to carry out the research work.

REFERENCE

1. J Y Reginster, *et al*, Strontium ranelate reduces the risk of non-vertebral fractures in women with osteoporosis: Treatment of peripheral osteoporosis (TROPOS) study. Journal of Clinical Endocrinology and Metabolism, 90 (5), 2816-2822, (2004).
2. Manette C, *et al*, comprehensive therapy in osteoporosis using a single drug: from ADFR to Strontium ranelate. Current chemistry, 13(13), 1585-1590(6), (2006).
3. http://www.lookchem.com/strontiumranelate/
4. http://www.chemicalbook.com/ChemicalProductPropertyENCB1838150/
5. http://en.wikipedia.org/wiki/Strontium_ranelate.
6. Yin Hong-Liu, *et al* HPLC determination of strontium ranelate and its related substances. Chinese Journal of Pharmaceutical analysis. R-97, CNKI: SUN: YWFX.02 (56), 1 (2009).
7. Dr P D Sethi, Quantitative analysis of Drugs in Pharmaceutical Formulations, 3rd Edn, 51-58. (2005).
8. ICH guidelines on analytical method validation, In: proceedings of the international conference of harmonization, Geneva, (1996). ICH Guidelines, Q2B, Analytical Method Validation.
9. N. Mallikarjun Rao, *et al,* RP-HPLC method development and validation for estimation of Voglibose in bulk and tablet dosage form. Int. J. Res. Pharm. Sci. 1(2), 190-194, (2010).
10. N. Sreekanth, *et al,* RP-HPLC method development and validation of Rimonabant Hydrochloride in bulk and pharmaceutical dosage form. International journal of pharmacy and pharmaceutical science, 1(1), 199-205, 12 (2009).