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A VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR THE DETERMINATION OF STRONTIUM RANELATE A DUAL ACTION BONE AGENT IN BULK AND SACHET DOSAGE FORM

B.R.C. Sekhar Reddy[1,*] and Nallagatla Vijaya Bhaskar Rao[2]
[1]Department of Chemistry, Acharya Nagarjuna University, Andhra Pradesh, India.
[2]Govt. Degree College, Alair, Nalgonda District, Andhra Pradesh, India.
*E-mail: brcsekharreddy@gmail.com

ABSTRACT
A reliable and sensitive isocratic stability indicating RP-HPLC method has been developed and validated for assay of Strontium Ranelate in tablets and for determination of content uniformity. An isocratic separation of Strontium Ranelate was achieved on Zodiac C-18 column (250mm x 4.6mm), 5 µm particle size columns with a flow rate of 1.1 ml/min and using a UV detector to monitor the eluate at 239nm. The mobile phase consisted of Methanol: Water: Acetonitrile in the ratio of 25:25:50 (v/v/v) (pH 4.7). Drug solution was subjected to oxidation, hydrolysis, photolysis and thermal degradation. All degradation products in an overall analytical run time of approximately 10min with the parent compound Strontium Ranelate eluting at approximately 4.83min. Response was a linear function of drug concentration in the range of 70-10 µg/ml (r^2= 0.999). Accuracy (recovery) was found to be well acceptance range of 98-102. Degradation products resulting from the stress studies did not interfere with the detection of Strontium Ranelate and the assay is thus stability-indicating.
Keywords: Strontium Ranelate, RP-HPLC, Forced degradation, Assay.

INTRODUCTION

Strontium is an element which was used in the past to treat osteoporosis. It fell out of use because it was associated with defects in bone mineralization. Strontium ranelate aims to overcome the problems associated with strontium. Strontium ranelate, a strontium (II) salt of ranelic acid, is a medication for osteoporosis. Studies indicate it can also slow the course of osteoarthritis of the knee. The drug is unusual in that it both increases deposition of new bone osteoblasts and reduces the resorption of bone by osteoclasts. It is therefore promoted as a "dual action bone agent" (DABA). Chemically it is called as distrontium 5-[bis(2-oxido-2-oxoethyl)amino]-4-cyano-3-(2-oxido-2-oxoethyl)thiophene-2-carboxylate[1].

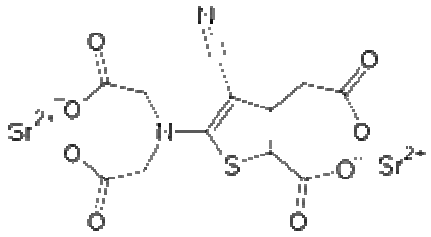

Fig.-1: Structure of Strontium Ranelate

Strontium ranelate is an anti osteoporotic agent which both increases bone formation and reduces bone resorption, resulting in a rebalance of bone turnover in favor of bone formation. This is similar to the effects of choline stabilized orthosilicic acid[2]. The possible side effects of Strontium ranelate are Nausea and diarrhoea. Headache, loose stools and eczema. Transient reversible increases in creatine kinase

activity[3]. Food, milk and derivative products, and medicinal products containing calcium may reduce the bioavailability of strontium ranelate.

Very few analytical methods have been reported for the estimation of Strontium ranelate in different forms using HPLC[4] and VU Visible spectrophotometry[5-7].

EXPERIMENTAL

Strontium Ranelate reference standard was provided by Glenmark PVT LTD, formulation sachetswere purchased from a local pharmacy. HPLC grade Methanol, Water and Acetonitrile were purchased from Merk specialties Pvt. Limited, Mumbai. 0.45µm nylon membrane filter papers were obtained from Pall Life Sciences, Mumbai.

Instrumentation

Chromatographic separation was performed on a PEAK chromatographic system equipped with LC-P7000 isocratic pump; Rheodyne injector with 20µl fixed volume loop, variable wavelength programmable UV detector UV7000 and the output signal was monitored and integrated by PEAK Chromatographic Software version 1.06. Teccomp UV-2301 double beam UV-Visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Sonicator (1.5L) Ultrasonicator was used to sonicating the mobile phase and samples. Standard and sample drugs were weighed by using Denver electronic analytical balance (SI-234) and pH of the mobile phase was adjusted by using Systronics digital pH meter.

Preparation of standard stock solution

Standard stock solution of Strontium Ranelate pure drug (1mg/ml) was prepared by accurately weighing about 100 mg of each drug in 100 ml volumetric flask. The drugs were dissolved with 25ml of methanol, and sonicated to dissolve it completely and made up to the mark with the same solvent. The contents were mixed well and filtered through Ultipor N_{66} Nylon 6, 6 membrane sample filter paper. Appropriate volumes of these solutions were further diluted with mobile phase to get required concentrations.

Preparation of sample solution

The contents of five sachets were mixed and weighed. The average weight of each sachet was found out. A quantity of sample equivalent to 10mg of strontium ranelate was weighed accurately and transferred into a clean 10 ml volumetric flask and dissolved in methanol solution. Then the solution was made up to the volume with mobile phase. The solution was sonicated for 15min and filtered through 0.45 µm membrane filter. Then the solution was further diluted with mobile phase to obtain the concentrations of 30µg/ml.

Method Development

For developing the method, a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wave length and choice of stationary and mobile phases. The following studies were conducted for this purpose.

RESULTS AND DISCUSSION

Detection wavelength

The spectrum of diluted solutions of the Strontium Ranelatein methanol was recorded. The absorption spectrum of Strontium Ranelate obtained by scanning the sample separately on UV spectrophotometer in UV region (200-400nm) in spectrum mode showed that the drug has maximum absorbance at 239nm. Analysis was carried out by adjusting the UV detector of the HPLC system at 239nm. Wavelength scanning spectra was shown in figure-2.

Choice of stationary phase

Preliminary development trials have performed with octadecyl columns with different types, configurations and from different manufacturers. Finally the expected separation and shapes of peak was succeeded Analytical column Zodiac C-18 column with 250 x 4.6mm internal diameter and 5µm particle size.

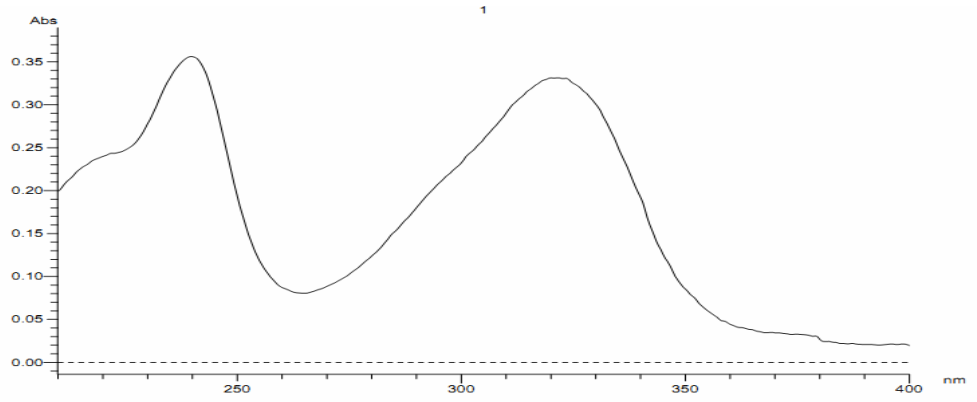

Fig.-2: Wavelength Scanning Spectra of Strontium Ranelate

Selection of the mobile phase

Based upon the solubility of the drug, initial developmental trails were performed on pure soluble solvents. It was found that Acetonitrile give comparatively best results than water and Methanol. Then different ratios with Acetonitrile as major solvent and Water, Methanol minor solvents. In the ratios broad peaks and low theoretical plates was observed. By change in different solvents, finally at a mobile phase Methanol: Water: Acetonitrile in the ratio of 25:25:50 (v/v/v) was found to be suitable. In this condition sharp peak was observed with high theoretical plates and less tailing factor. The optimized mobile phase pH was found to be 4.7.

Selection of the mobile phase flow rate

Flow rates of the mobile phase were changed from 0.8 – 1.2 ml/min for optimum separation. A minimum flow rate as well as minimum run time gives the maximum saving on the usage of solvents. It was found from the experiments that 1.1ml/min flow rate was ideal for the successful elution of the analyte.

Optimized conditions

After completion of the developmental conditions, the conditions were validated for the acceptance of the developed method. The optimized conditions were shown in table-1.

Method Validation

Specificity

Placebo of the tablets, equivalent to the sample weight was taken and solution prepared similarly to the sample solution. The solution was analyzed as per the proposed method. Sample solution was also analyzed as per the proposed method. No interference from placebo was observed at the retention time of the drugs peaks. Peak purity plots also indicated that the peaks of Strontium Ranelate are pure and don't have any co eluting peaks. Therefore, it is concluded that the method is specific.Specificity chromatogram was shown in figure-3

Linearity and range

Standard stock solution was selectively diluted to get different concentration ranges. The prepared solutions were injected into HPLC system. It was found that linear regression was obtained within the concentration range of 10-70µg/ml. The linearity was evaluated by linear regression analysis using the

least square method. It was found that correlationcoefficient and regression analysis are within the limits. Linearity graph was shown in figure-4.

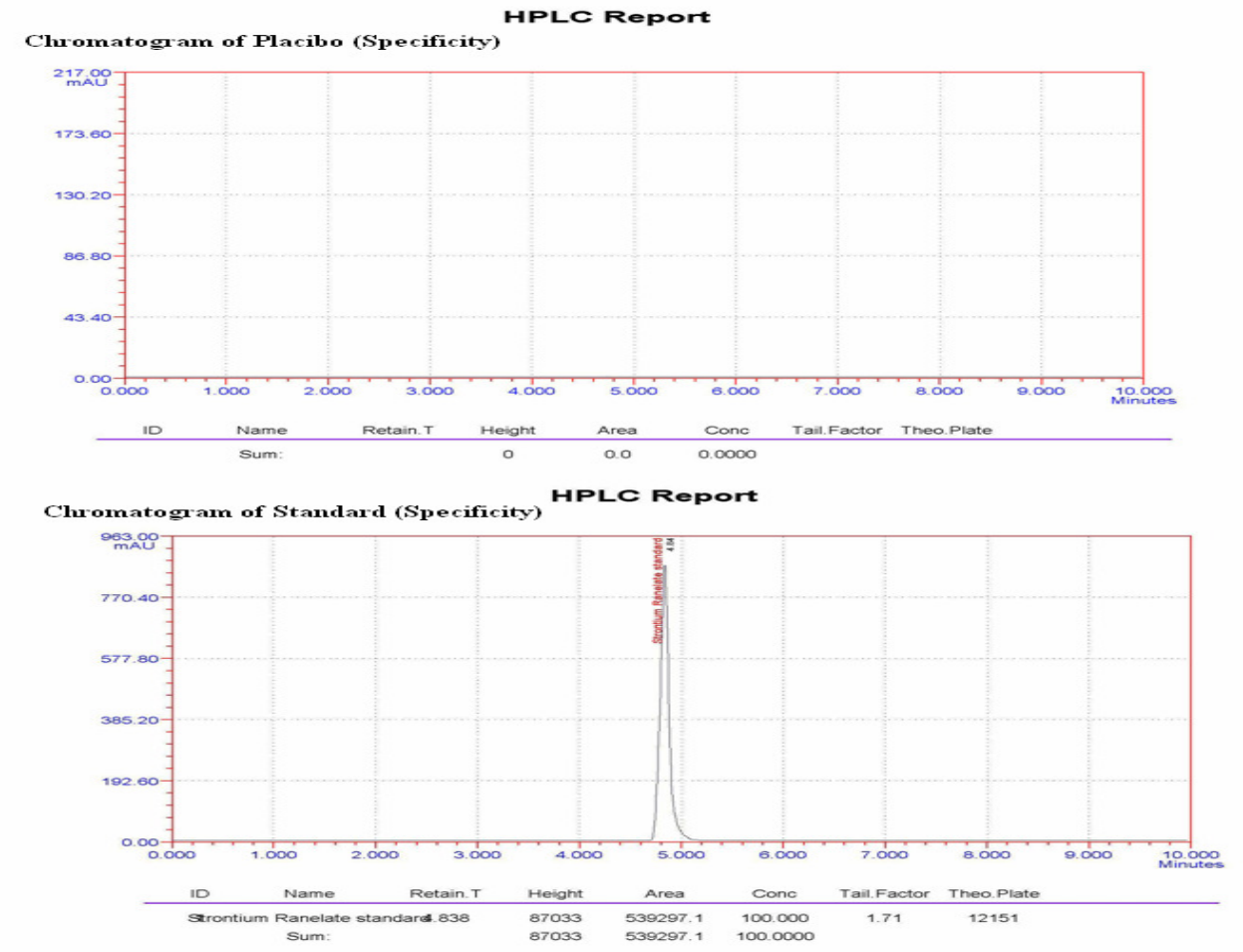

Fig.-3: Specificity chromatogram of Strontium Ranelate

Table-1: Optimized chromatographic conditions of Strontium Ranelate

Parameter	Condition
Mobile Phase	Methanol: Water: Acetonitrile 25:25:50(v/v/v)
Pump mode	Isocratic
pH	4.7
Diluent	Methanol
Column	Zodiac C18 column (250 X 4.6 mm, 5µ)
Column Temp	Ambient
Wavelength	239nm
Injection Volume	20 µl
Flow rate	1.1m/min
Run time	10minutes
Retention Time	4.84minits
Pump Pressure	6.5±5MPa

Precision

The precision of the assay was determined in terms of intra-day and inter-day precision. The intra-day and inter-day variation in the peak area of drug solution was calculated in terms of % Relative standard deviation (%RSD) obtained by multiplying the ratio of standard deviation to mean with 100.% RSD was found to be 0.27 for intraday and 0.67for interday precision, which found to be under the acceptance criteria.

Recovery

Accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. The standard addition method was performed at 50%, 100% and 150% levels of standard 20µg/ml. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and % RSD was calculated and results are presented in Table-2. Satisfactory recoveries ranging from 98.10 to 101.77 were obtained by the proposed method. The values of recovery justify the accuracy of the method. The % recovery values were obtained within the standard limit which confirms that the method is accurate and free from any positive or negative interference of the excipients. This indicates that the proposed method was accurate.

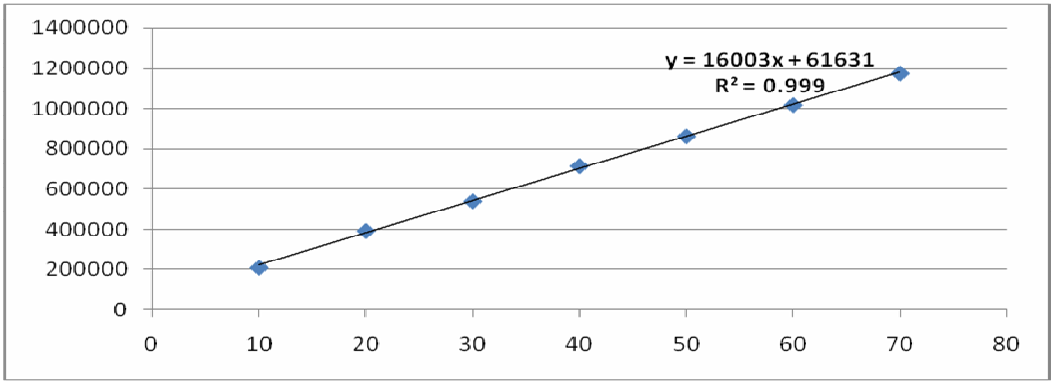

Fig.-4: Linearity graph of Strontium Ranelate

Table-2: Recovery results of StrontiumRanelate

Level	Target µg/ml	spiked (µg/ml)	Total in µg/ml	Amount recovered(µg/ml)	% Recovery	%RSD of Recovery
50 %	20	10	30	29.43	98.10	0.67
	20	10	30	29.7	99.30	
	20	10	30	29.46	98.21	
100%	20	20	40	39.74	99.3	0.92
	20	20	40	39.43	98.59	
	20	20	40	40.19	100.4	
150%	20	30	50	50.35	100.71	0.64
	20	30	50	50.31	100.6	
	20	30	50	50.88	101.77	

Robustness and Ruggedness of the method

Robustness of the method was confirmed by small deliberate variations in the method conditions and theresults obtained in the changed condition were compared with the standard values. Here Robustnesswas tested by ±5% changein the mobile phase ratio, wavelength of the detector and the pH of the detector at a concentrationof 30µg/ml solution. It was found that that there is no remarkable change in

the results with all the changed conditions. This indicates that the method is robust. Ruggednesswas tested by change in the different persons in three different days at 30µg/ml concentrations and %RSD was calculated and was found to be 1.20, which is under the acceptance criteria. Summary results were shown in table- 3.

Solution stability

Solution at standard concentration was prepared and this was incubated at room temperature and the stability of the solution was tested periodically. It was found that there is no remarkable change in the results was found 24hours.

Forced degradation studies

To perform the forced degradation study 50 mg drug was subjected to acidic, alkaline, oxidizing, thermal and photolytic conditions. For acidic degradation the drug was heated under reflux with 0.1 M HCl at 80º for 2 h and the mixture was neutralized. For alkaline degradation the drug was treated with 0.1 M NaOH at 80º for 2 h and the mixture was neutralized. For degradation under oxidizing conditions the drug was heated under reflux with (30%, v/v) H_2O_2 at 80º for 2 h. For thermal degradation the powdered drug was exposed at 70º for 48 h. For photolyticdegradation the powdered drug was exposed to sunlight for 48 h. The placebo was also subjected to the same stress conditions to determine whether any peaks arose from the declared excipients. After completion of the treatments the solutions were left to return to room temperature and diluted with solvent mixture to furnish 30µg/ml solutions. The purity of the drug peak obtained from the stressed sample was measured UV detector and compares the chromatogram of untreated drugs in tablet solution. It was found that there is no degradation and changes in the unstressed sample and comparatively very less changes in the stressed samples indicating that the method is stable under the studied conditions. Chromatograms were shown in figure-5.

Formulation analysis

The prepared sample solution was injected into HPLC system. Peak area response was used to determine the %assay found in the formulation was analyzed. It was found that 99.17% assay was found in the commercial formulation. Results were shown in table-4.

Table-4: Formulation results of Strontium Ranelate

S.No.	Brand Name	Strength	Concentration	Amount found	%Assay
1	Stronat	2g/Sachet	30µg/ml	29.75µg/ml	99.17

Table-3: Summary results of Strontium Ranelate

S.No.	Condition	Result
1	Linearity Range	10-70µg/ml
	Slope	16003
	Intercept	61631
2	Precision (%RSD)	
	Intraday	0.27
	Interday	0.67
3	Ruggedness (%RSD)	1.20
4	Robustness (% Change)	
	MP change - 1	1.28
	MP change -2	1.42
	WL change-1	0.24
	WL change-2	1.34
	pH-1	0.5
	pH-2	0.87

5	Limit of Detection	0.075µg/ml
6	Limit of Quantification	0.25µg/ml
7	Solution stability (time in Hours)	Stable up to 24H
8	Formulation (%Assay)	99.17

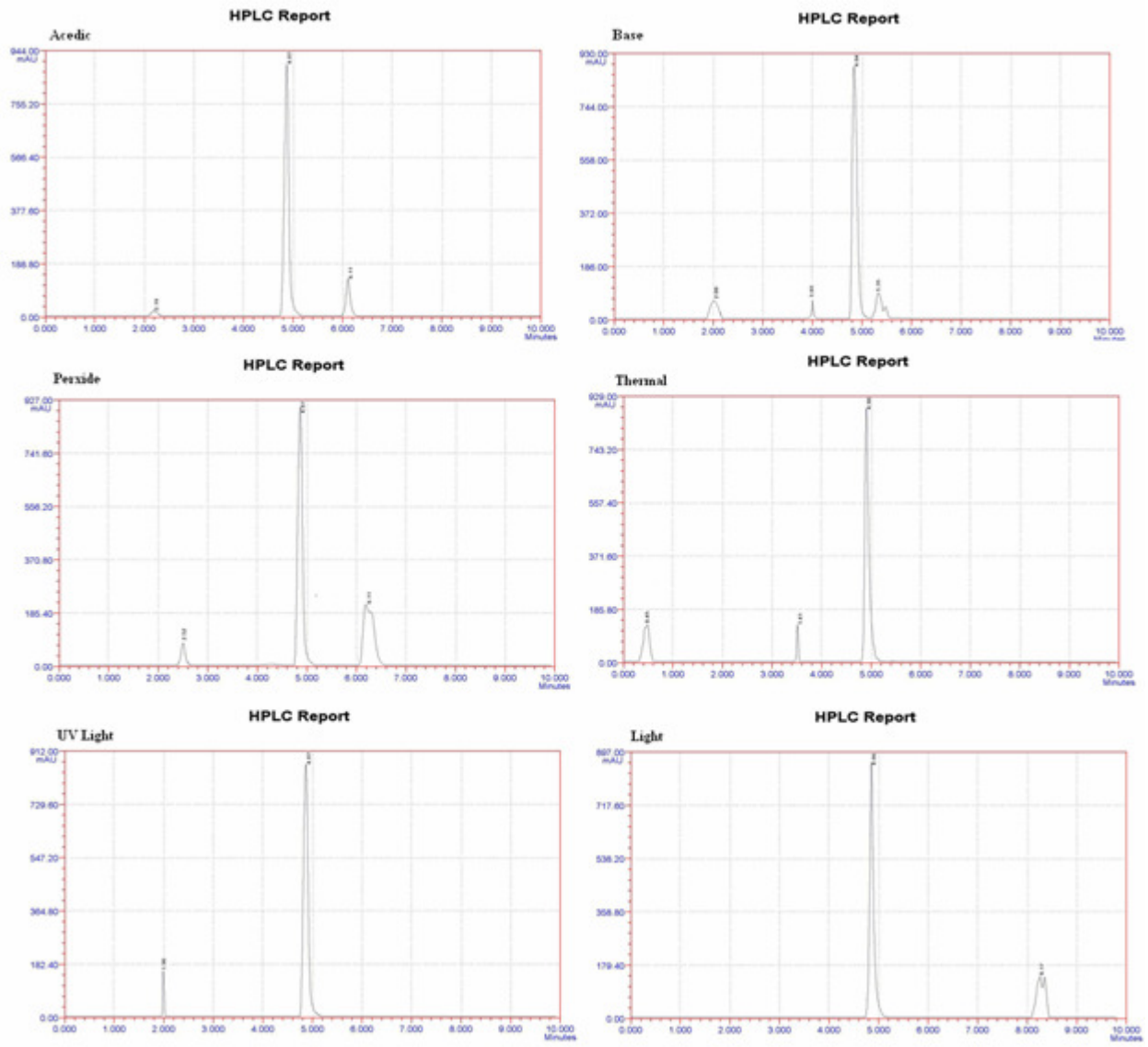

Fig.-5: Forced degradation study chromatograms of Strontium Ranelate

CONCLUSION

The intensive approach described in this manuscript was used to develop and validate a liquid chromatographic analytical method that can be used for both assay and determination of content uniformity of Stronium Ranelate in a pharmaceutical dosage form. Degradation products produced as a result of stress did not interfere with detection of Stronium Ranelate and the assay method can thus be regarded as stability indicating. This HPLC method for assay and determination of content uniformity of Stronium Ranelatein a tablet formulation was successfully developed and validated for its intended purpose. The method was shown to specific, linear, precise, accurate, and robust. Because the method separates Stronium Ranelate and all the degradation products formed under variety of stress conditions it

can be regarded as stability indicating. Because there is no pharmacopeial method for assay and determination of content uniformity of Stronium Ranelate in pharmaceutical dosage forms, this method is recommended to the industry for quality control of drug content in pharmaceutical preparations.

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