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DEVELOPMENT AND VALIDATION OF A HPTLC METHOD FOR DETERMINATION OF STRONTIUM RANELATE IN THE PRESENCE OF ITS IMPURITIES

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ABSTRACT

Impurity profiling has gained importance in modern pharmaceutical analysis due to the fact that an unidentified, potentially toxic impurity are hazardous to health and in order to increase the safety of drug therapy, impurities should be identified and determined by selective methods. Thus a simple sensitive high performance thin layer chromatographic (HPTLC) method for estimation of Strontium ranelate and its impurities has been developed. Strontium ranelate and its impurities were separated and identified on silica Gel 60 F_{254}TLC plates with Acetonitrile : Methanol: Ethyl acetate: Formic acid 9:1:1:1 *(v/v)* as mobile phase. The quantification was performed at λ = 320 nm. The method was validated for linearity, accuracy, precision and robustness according to ICH guidelines. The calibration plots were linear in the range 4000-9000 and 40-90 ng/band for API and its two impurities respectively. The developed method can be used as a quality control tool for Strontium ranelate impurity profile study.

KEY WORDS: Strontium Ranelate (STR), High performance thin layer chromatography (HPTLC), Analytical Method Validation, Impurity Profile, Method Optimization

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INTRODUCTION

Various regulatory authorities focus on the control of impurities: the International Conference on Harmonization (ICH), the United States Food and Drug Administration (USFDA), the European Medicines Agency (EMA), the Canadian Drug and Health Agency, the Japanese Pharmaceutical and Medical Devices Agency (PMDA), and the Australian Department of Health and Ageing Therapeutic Goods. In addition, a number of official compendia, such as the British Pharmacopoeia (BP), the United States Pharmacopeia (USP), the Japanese Pharmacopoeia (JP), and the European Pharmacopoeia (EP) are incorporating limits that restrict the impurity levels present in APIs as well as in drug formulations. Impurities must be monitored carefully to assure the quality of drugs. It is important to identify potential sources of such impurities. Hence selective analytical methods need to be developed to monitor them[1]. Strontium ranelate,(STR) a strontium (II) salt of ranelic acid, (5-[Bis (carboxymethyl) amino] 2-carboxy-4-cyno-3-thiopheneacetic acid strontium salt (1:2), octahydrate) (Figure 1); is a medication for osteoporosis[2]. 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). The most common side effects include nausea, diarrhea, headache and eczema. Strontium, which has the atomic symbol Sr and the atomic number 38, belongs to the group II in the periodic table of the elements, just beneath calcium. Because its nucleus is very nearly the same size as that of calcium, the body easily takes up strontium and incorporates it into bones and tooth enamel in the place of calcium[3].

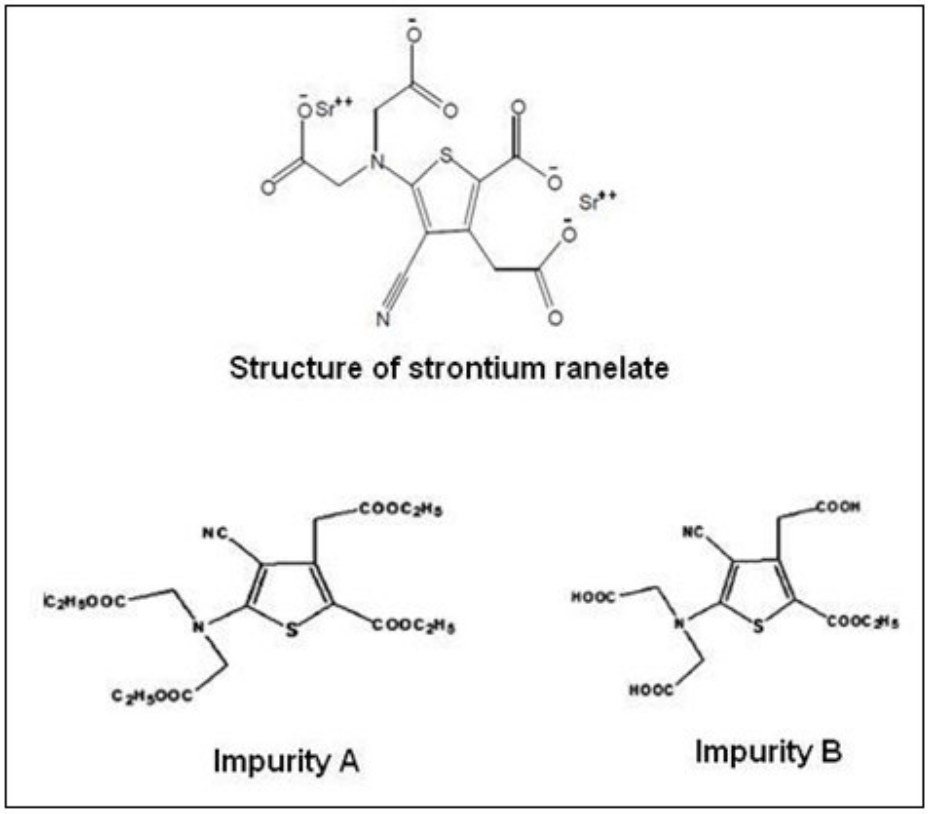

Figure 1
structure of Strontium ranelate and its two impurities

Literature survey revealed that different analytical methods for the determination of Strontium ranelate have been reported, which include UV spectrophotometric method[4,5], high performance liquid chromatography (HPLC) method for determination of API from formulation[6] and a stability indicating LC method for quantification[7,8]. However no HPTLC method has been reported for determination of Strontium ranelate and its two impurities named Impurity A: Tetraethyl Ranelate 5-[bis (2-ethoxy-oxoethyl) amino]-4-cyno-2-(ethoxycarbony thiopheacetic acid ethyl ester, Impurity B**:** 2, 2'-{[4-(carboxymethyl)-3-cyno-5-(ethoxycarbonyl)-2-thienyl] imino} diacitic acid[9] (Figure. 1). As compared to the other analytical techniques, HPTLC is the most simple and less time consuming technique. This technique has an advantage that one can perform many parameters at one time, which is not possible with other techniques[10]. Hence this article describes a simple, precise, accurate validated HPTLC method for quantitative estimation of the API and its impurities.

MATERIALS AND METHODS

Materials & Reagents

Strontium ranelate pure analytical sample was obtained as a gift sample from Glenmark Pharmaceutical Ltd., Nashik and its impurities; impurity A and impurity B were procured from Sigma-Aldrich Ltd. Strontium ranelate commercially available as granules in sachet with the Brand name 'Stronat' containing Strontium ranelate 2 g per sachet, Batch No. O1132405, manufactured by Glenmark Pharmaceutical Ltd., Nashik, India were purchased locally. All chemicals used were of Analytical reagent grade including acetone, acetonitrile, methanol, hexane, ethyl acetate and formic acid purchased from Research Lab Fine Chem Industries, Mumbai.

Standard and Sample preparation

Standard Solution

Standard stock solution containing 10000 µg/mL of Strontium ranelate was prepared in methanol by weighing 100 mg of API dissolving it in sufficient quantity of methanol and finally volume was made up to 10 mL with methanol. Similarly working standard for the two impurities, i.e impurity A and impurity B was prepared in methanol having concentration 10 µg/mL.

Mixed standard solution

From the above standard solution of Strontium ranelate (10000 µg/mL), 1 ml was taken and to it 1 ml of stock solution (10 µg/mL) of each impurity was added and the volume was made upto 5 mL with methanol to get the mixed standard solution of API and impurities. From this solution equivalent volume was spotted on plate to give the desired concentration of API and impurities.

Sample Solution

Five sachets of 'Stronat' were emptied into a mortar pestle, triturated it lightly and weighed. The quantity of powder equivalent to 100 mg was weighed and transferred to 100 mL volumetric flask. The powder was dissolved in methanol by sonication for 5 min; the solution was filtered through a whatmann filter paper no. 41 and finally volume was made up to the mark with methanol. Further from the above stock solution 5 mL was diluted to 50 mL with methanol to get the solution containing 100 µg/mL of Strontium ranelate.

Chromatography

Chromatography was performed on 20 x10 cm aluminium backed silica gel 60 F_{254}TLC plates (Merck, Darmstadt, Germany). Before use, the plates were washed with methanol and activated at 110 ^{0}C for 5 min. Acetonitrile: Methanol: Ethyl acetate: Formic acid 9:1:1:1 *(v/v)* were used as the mobile phase. Samples (5 µL) were applied to the plates as 6 mm bands, keeping 17.7 mm distance between bands and 8 mm distance from plate side edge and 8 mm distance from bottom of plate. CAMAG (Muttenz Switzerland) Linomat V was used as sample applicator equipped with 100 µL syringe (Hamilton, Reno, Nevada, USA) with the spraying rate as150 nL/sec. The plates were developed in CAMAG 20 x 10 cm twin trough chamber lined with filter paper and saturated with mobile phase vapors for 10 min

at room temperature. The solvent front position was set as 80 mm. After development of the plate in the mobile phase, it was dried in hot air oven at 50^0 C for 5 min. Densitometric scanning of the developed plate was performed at wavelength λ= 320 nm using CAMAG TLC scanner 3 with win CATS Software and deuterium lamp as light source with the slit dimension 4.00 x 0.30 mm and scanning speed of 20 mm/s.

Optimization of Method

HPTLC procedure was optimized to develop a method for identification and quantification of drug and its two impurities simultaneously. Based on the literature survey and the polarity of the STR, initially individual solvents were tried as mobile phase, like Hexane, ethyl acetate, methanol. From the results of above trial, the mobile phase composition containing Hexane: Ethyl acetate (5:5) *v/v*; was tried. In this mobile phase the bands were observed near to the spotting line. Therefore to increase the polarity of mobile phase, methanol was added to the above composition i.e mobile phase containing Hexane: ethyl acetate: Methanol (6:2:0.5 *v/v*) was tried. In this also the STR and impurities bands were not moving at all. Hence to get resolved bands of impurities, polarity of the solvent in the mobile phase has to be increased hence Hexane was removed i.e Ethyl acetate: methanol (5:5) *v/v* was used as the mobile phase. But in this case STR band was moving but very short distance and impurities were not at all resolved. Finally it was concluded that, the mobile phase containing solvents which has the polarity more than hexane as well as methanol must be used to get the proper resolution of STR and impurities bands. Hence mobile phase containing Acetonitrile: Methanol: Ethyl acetate (6:4:0.5 *v/v*) was tried, in this all three bands were resolved but not to a considerable limit. Hence again the composition of the mobile phase was changed to Acetonitrile: Methanol: Ethyl acetate (8:1.5:0.5 *v/v*); here all the bands were well resolved but the peaks were having tailing and fronting. Hence finally mobile phase containing Acetonitrile: Methanol: Ethyl acetate: Formic acid 9:1:1:1 *(v/v)* was tried to reduce the tailing effect. This mobile phase showed well resolved peaks of drug and its two impurities with the reproducible results and the method was said to be optimized.

Method Validation

The method was validated according to ICH guidelines for the parameters including accuracy, precision, linearity, specificity and robustness in analytical solution[11, 12, 13].

Linearity

The equivalent volume of mixed standard solution (2.0-4.5 µL) was spotted on plate to cover the range of 4000-9000 ng per band and 40 to 90 ng per band of standard drug and its two impurities respectively. Chromatographic plates were developed using optimized procedure and scanned as described above. The peak areas were recorded for Strontium ranelate and its impurities. A good linear relationship between response (peak area) and concentration was obtained. As the calibration can be accepted as linear, only if it is proven statistically hence to prove the linearity of the data; non numerical test method (testing the residuals) was performed[14, 15, 16].

Method sensitivity (LOD and LOQ)

To calculate the LOD and LOQ of the method, the calibration curve of API and its two impurities was performed thrice (n=3). From the above data, standard deviation and the slope was calculated. All this data was processed as per the formula for limit of detection LOD and limit of quantitation LOQ given as LOD= 3.3x σ/S and LOQ=10x σ/S, where σ is standard deviation and S is slope of calibration plot.

Assay

The concentration selected to perform assay was 4000 ng/band. The area of this solution was measured 6 times and % assay was calculated. The area under curve for the sample solution was compared with the data of calibration curve and the % assay was calculated.

Accuracy (Recovery)

The accuracy of the method was determined by calculating recoveries of Strontium ranelate and its impurities by the standard addition method. Recovery was performed at three levels i.e 80, 100 and 120 %. Known amount of standards as 3200, 4000 and 4800 ng per band were spiked to the sample band (4000 ng). Similarly known amount standard impurities (32, 40, 48 ng per band) were spiked to the sample solution of impurities (40 ng). The amount of Strontium ranelate and its impurities were determined by measuring the peak areas.

Precision

For the precision both repeatability and intermediate precision of the method for drug and its impurities was checked. For repeatability the peak area of sample band was measured repeatedly (n=6) and the % relative standard deviation (% RSD) was calculated. For intermediate precision, the peak area of sample at three different concentrations were measured for three times (n=3) on the same day for intraday precision and on three different days for inter day precision and the % relative standard deviation (% RSD) was calculated.

Robustness

Robustness of the method was evaluated by deliberately changing the parameters which include volume of mobile phase, saturation time, composition of mobile phase, TLC plate form different lot and time from spotting to development and development to scanning. By changing the above parameters the peak area of the API and its impurities were recorded. Robustness of method for each parameter change was assessed by using % assay of analyte.

Specificity

The specificity of the method was determined by analyzing standard drug, test sample and its two impurities. The spot for Standard, sample drug and impurities was confirmed by comparing the R_f and spectrum of the spot to that of a standard. The peak purity was determined by comparing R value of the spectrum at different regions of the spot, i.e, peak start (S) to peak apex (M) and peak apex (M) to peak end (E).

RESULTS AND DISCUSSION

Linearity

Calibration curves were obtained for strontium ranelate and its two impurities from which linear regression equation was computed and a correlation coefficient was obtained. Linear regression data for the calibration plots (n = 3) are listed in (Table 1). The correlation coefficient was found to be in the range of 0.997-0.999 for all the components, which shows excellent linearity between the concentration and response. From non numerical test; the plot of residuals was obtained which doesn't show any trend in it that implies results are satisfactory. This proves the linearity of calibration.

Table 1

Linear Regression data for the calibration plot (n=3) and Method Sensitivity

	STR	Impurity A	Impurity B
Linearity Range (ng)	4000-9000	40-90	40-90
Correlation Coefficient	0.999	0.997	0.998
Slope	16.736	14.1	6.038
Intercept	19114	368.4	377.2
LOD (ng)	429.3897	8.598895	3.731694
LOQ (ng)	1301.181	26.05726	11.30816

Method sensitivity (LOD and LOQ)

The LOD and LOQ were found to be 429.3 ng and 1301.1 ng for API and 8.59 and 26.05 ng for Impurity A, and 3.73, and 11.30 ng for impurity B respectively (Table 1). The low values of LOD and LOQ reveals that the method is sensitive.

Assay

The drug content obtained from standard and the sample was found to be comparable with no interference from the excipients commonly present. The R_f value was found to be 0.43 and the assay value was found to be 99.89 % and % RSD value as 0.65. The result indicates that the method is suitable for routine analysis of STR and its impurities in pharmaceutical dosage form.

Accuracy

The % recovery was found in the range of 99.09–101.02% and % RSD value was less than 2 for STR and its two impurities (Figure 2). The result shows that the method is accurate (Table 2).

Table 2
Recovery Study (n=6)

Spike level	% Recovery			% RSD		
	STR	Impurity A	Impurity B	STR	Impurity A	Impurity B
80 %	99.09	99.94	99.71	0.65	0.1	0.46
100 %	99.23	101.02	99.8	0.16	0.291	0.11
120 %	100.03	99.82	99.88	0.54	0.105	0.11

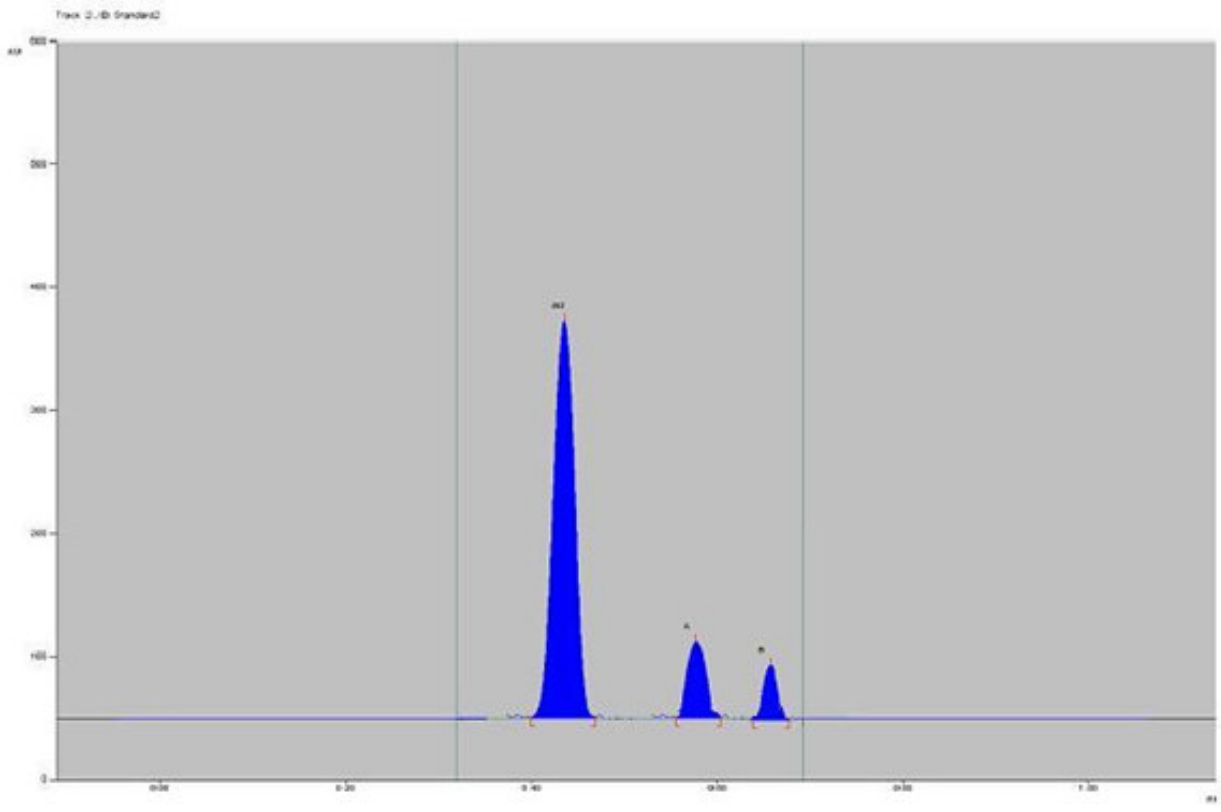

Figure 2
Densitogram of STR and its two impurities

Precision

To check precision of the method, the response of sample solution was recorded three times for three different concentrations. The intraday and inter day precision was performed as per the procedure given in previous section. The results are as shown in (Table 3).

Table 3
Precision Study (n=3)

Concentration	STR			Impurity A			Impurity B		
(ng/band)	4000	5000	6000	40	50	60	40	50	60
Intra day (% RSD)	0.301	0.456	0.378	1.16	1.05	1.03	0.153	0.195	0.205
Inter day (% RSD)	0.925	0.897	0.952	0.203	0.552	0.628	0.108	0.506	0.458

Robustness

Statistical analysis reveals that there is no significant difference between results obtained by applying some deliberate changes in the experimental conditions; thus this method was found to be robust. Table 4 shows the result of robustness study.

Table 4
Results of Robustness study

Parameter		STR % Assay, % RSD	Impurity A % Assay, % RSD	Impurity B % Assay, % RSD
Volume of mobile phase (±1 mL)		100.45, 0.97	99.67, 1.12	99.70,1.02
Time of saturation (±5 min)		100.6, 1.09	100.5, 0.98	99.85, 1.25
Composition of mobile phase Acetonitrile (±0.1 mL)		100.85, 1.4	101.2, 1.6	99.58, 1.5
Time from (± 5 min)	application to chromatography	101.4, 1.05	101.02, 0.85	99.45, 0.99
	chromatography to scanning	99.67, 1.23	101.07, 1.7	99.25, 1.2
Plate from different lot:	Lot HX007719	100.19, 0.99	100.16, 0.97	101.2,0.95
	Lot HX007915	100.01, 1.26	100.32,1.2	99.39, 1.03

Specificity

Well resolved peaks were obtained for STR and its two impurities (Figure 3). The values of peak purity are found to be in the range of 0.999730 to 0.999998 which shows that the method is specific for the determination of impurities and drug simultaneously. Table 5 shows the values of peak purity.

Table 5
Peak purity values

	STR	Impurity A	Impurity B
r,s,m	0.999951	0.999730	0.999998
r,m,e	0.999766	0.999954	0.999816

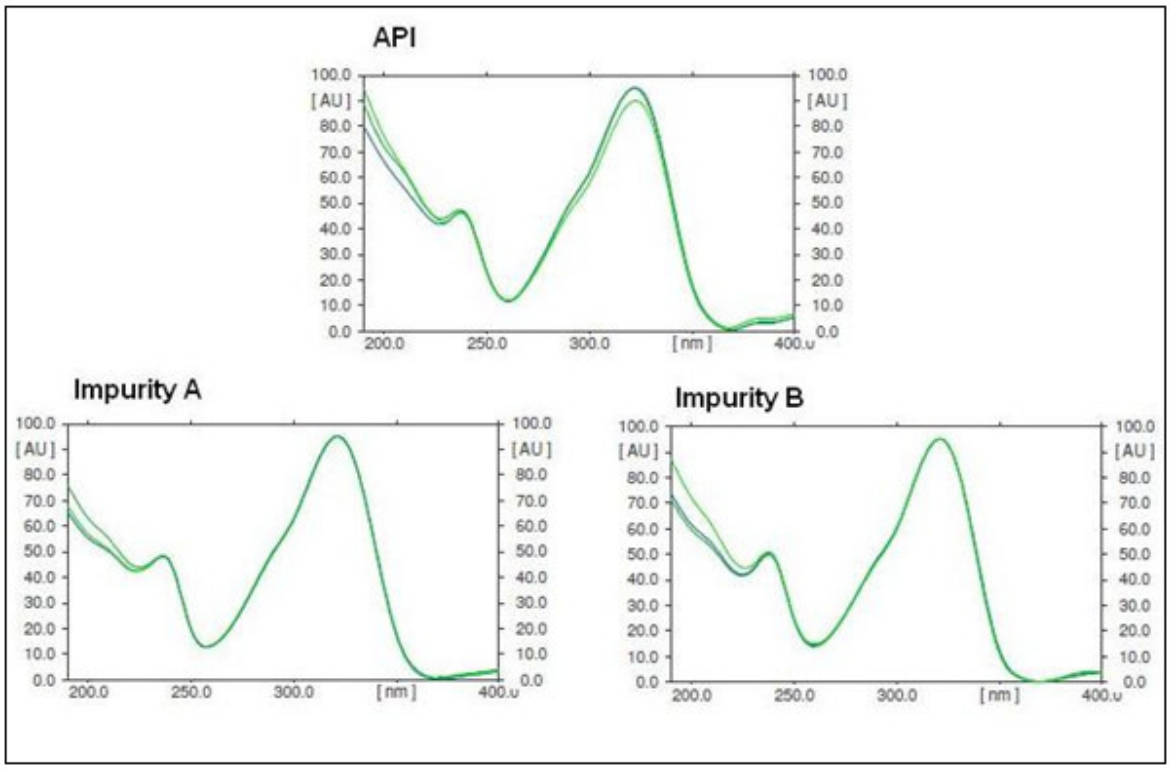

Figure 3
Overlain UV spectra of STR and its impurities for peak purity

CONCLUSION

HPTLC technique is most suited for impurity profile of drug substances as per the compendial specifications. This technique also gives extreme flexibility in various steps including selection of stationary phase, mobile phase, developing technique etc. Hence a simple, Specific, accurate, precise, robust and sensitive HPTLC method is developed. This method can be used in routine analysis of Strontium ranelate in presence of its impurities which will serve as a quality control tool for the routine analysis of STR and formulation.

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