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Development and Validation of Stability Indicating HPTLC Method for Estimation of Seratrodast

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Abstract*: A simple, sensitive, and accurate stability indicating HPTLC method has been developed and validated for estimation of Seratrodast in bulk and pharmaceutical dosage form. The drug was spotted on precoated silica gel 60 F_{254} aluminum plates using Toluene: Methanol: Glacial acetic acid (8.5:1:0.5 v/v/v) as mobile phase. The retention factor (R_f) was found to be 0.52 ± 0.04. The detection of band was carried at 266 nm. The drug was subjected to different stress conditions like acid, base, neutral hydrolysis, oxidation, thermal degradation and photolysis. The method was successfully validated according to ICH guidelines Q2 (R1). The data of linear regression analysis indicated a good linear relationship over the range of 100-600 ng/band concentrations with correlation coefficient 0.994. The accuracy of the method was established based on the recovery studies.*

The developed method was found to be simple, sensitive, selective, accurate, and repeatable for analysis of Seratrodast and can be adopted for routine analysis of drug in bulk and pharmaceutical dosage form.

Keywords: *High performance thin layer chromatography (HPTLC), Seratrodast, Stability indicating, Validation.*

1. INTRODUCTION

Seratrodast chemicaly is 7-phenyl-7-(2, 4 ,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl) heptanoic acid. It is used in treatment of asthma [1]. Literature survey reveals methods reported viz simple HPLC [1-2] and Bioanalytical HPLC method [3] for estimation of Seratrodast.

To the best of our knowledge no stability indicating HPTLC method has been reported for estimation of Seratrodast. The present work describes a simple stability indicating HPTLC method for the determination of Seratrodast in bulk and pharmaceutical dosage form (Seretra -80) according to the International conference on harmonization (ICH) guidelines. [4-5]

2. MATERIALS AND METHODS

Reagents and chemicals:

Authentic sample of Seratrodast was obtained from Vrgo Labs, Goa. The formulation Seretra – 80 labeled to contain Seratrodast 80 mg was procured form local market. Methanol (AR grade), Toluene (AR grade) were purchased from S. D. Fine Chemical Laboratories, Mumbai. Hydrochloric acid (HCl), hydrogen peroxide (H_2O_2) and sodium hydroxide (NaOH), Glacial acetic acid were purchased from LOBA CHEMIE PVT. LTD., Mumbai.

Chromatographic condition:

Chromatographic separation of drug was performed on aluminum plates precoated with silica gel 60 F_{254}, (10 cm × 10 cm with 250 µm layer thickness). Sample was applied on the plate as a band of 4 mm width using Camag 100 µl sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (Camag, Switzerland). The mobile phase was composed of Toluene: Methanol: Glacial acetic acid (8.5:1:0.5 v/v/v). 10 cm × 10 cm CAMAG twin trough glass chamber was used for linear ascending development of TLC plate under 15 min saturation conditions and 10 ml of mobile phase was used per run, migration distance was 80 mm. Densitometric scanning was performed using Camag TLC

scanner 3 in the range of 400-200 nm, operated by winCATS software, slit dimensions were 3.00 x 0.45 mm and Deuterium lamp was used as a radiation source.

Selection of Detection Wavelength

From the standard stock solution (1000 µg/ml) further dilutions were made using methanol and scanned over the range of 200 - 400 nm and the spectra was obtained. It was observed that the drug showed considerable absorbance at 266 nm.

Preparation of Standard stock solution

Standard stock solution of drug was prepared by dissolving 10 mg of the drug in 10 ml of methanol to get concentration of 1000 µg/ml. From the standard stock solution, working standard solution was prepared containing 100 µg/ml of Seratrodast. 5 µl of the resultant solution was applied on TLC plate to get concentration of 500 ng/band. Representative densitogram of Seratrodast (500 ng/band) is shown in Figure 1.

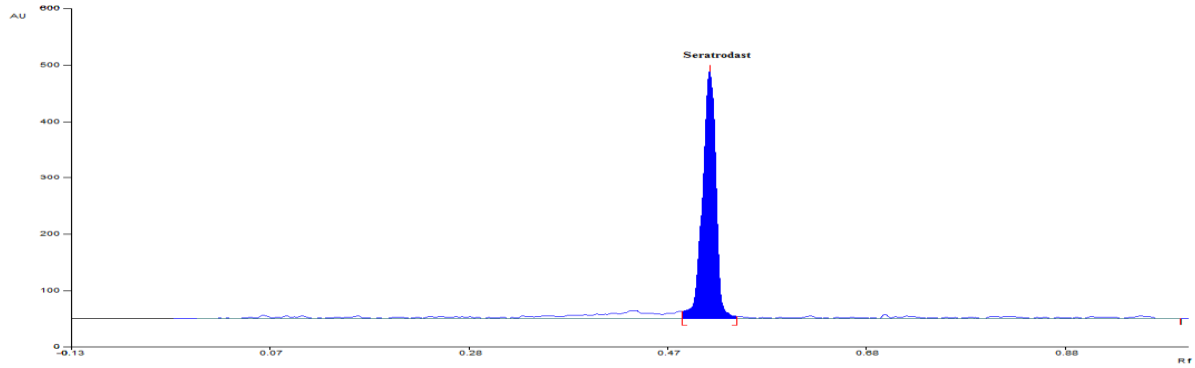


Figure 1. *Representative densitogram of Seratrodast (500 ng/band)*

Preparation of sample solution

A tablet containing 80 mg of Seratrodast (Seretra-80) was weighed and powdered. Powder equivalent to 10 mg of drug was transferred to 10 ml volumetric flask and volume was made up with methanol to get concentration (1000 µg/ml) and was sonicated for 10 min. Solution was filtered, 1 ml of filtrate was diluted to 10 ml with methanol. 2 µl of the resultant solution was applied on TLC plate to get concentration of 200 ng/band.

Stress degradation studies of bulk drug

Stability studies were carried out to provide evidence on how the quality of drug varies under the influence of a variety of environmental conditions like acidic, alkaline, hydrolysis, oxidation. Dry heat and photolytic degradation were carried out in the solid state. The degradation product was not seen at concentration of 500 ng/band; So 2000 ng/band concentration was applied for each stress condition. The degradation product was observed under alkaline condition only.

Alkaline hydrolysis

To 1 ml of 1000 µg/ml solution of Seratrodast, 1ml of 0.01 N NaOH was added. The above solution was kept for 4 hours at room temperature. The volume was made up to 10 ml with methanol. 5µl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 76.52 % of Seratrodast was recovered with one peak of degradation at R_f 0.27. Representative densitogram obtained for sample subjected to alkaline hydrolysis is shown in Figure 2

Acid hydrolysis

To 1 ml of 1000 µg/ml solution of Seratrodast, 1ml of 0.01 N HCl was added. The above solution was kept for 1 hour at room temperature. The volume was made upto 10 ml with methanol. 5 µl of the resultant solution was then applied at TLC plate and densitogram was developed. 73.34% Seratrodast was recovered with no peak of degradant. Representative densitogram obtained for sample subjected to acid hydrolysis is shown in Figure 3 a.

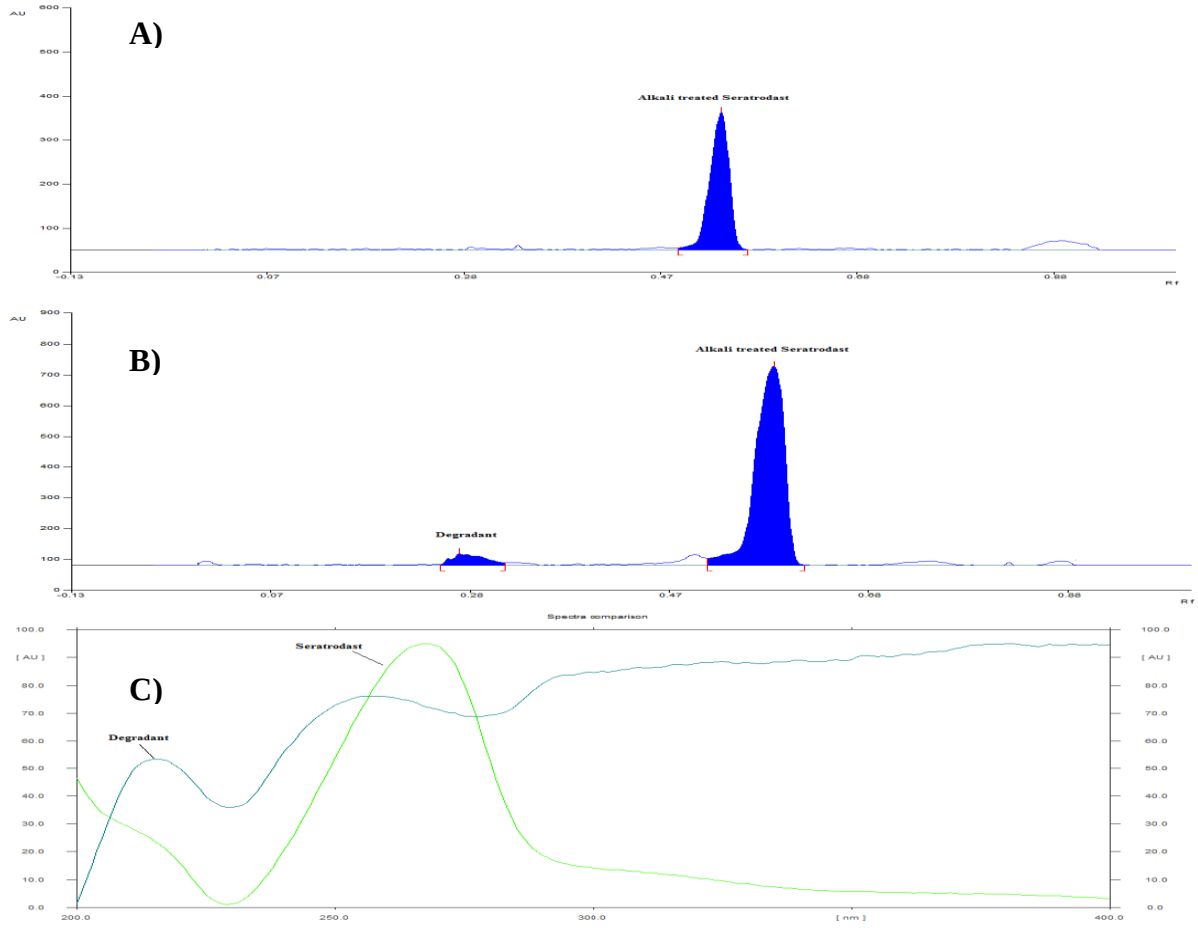
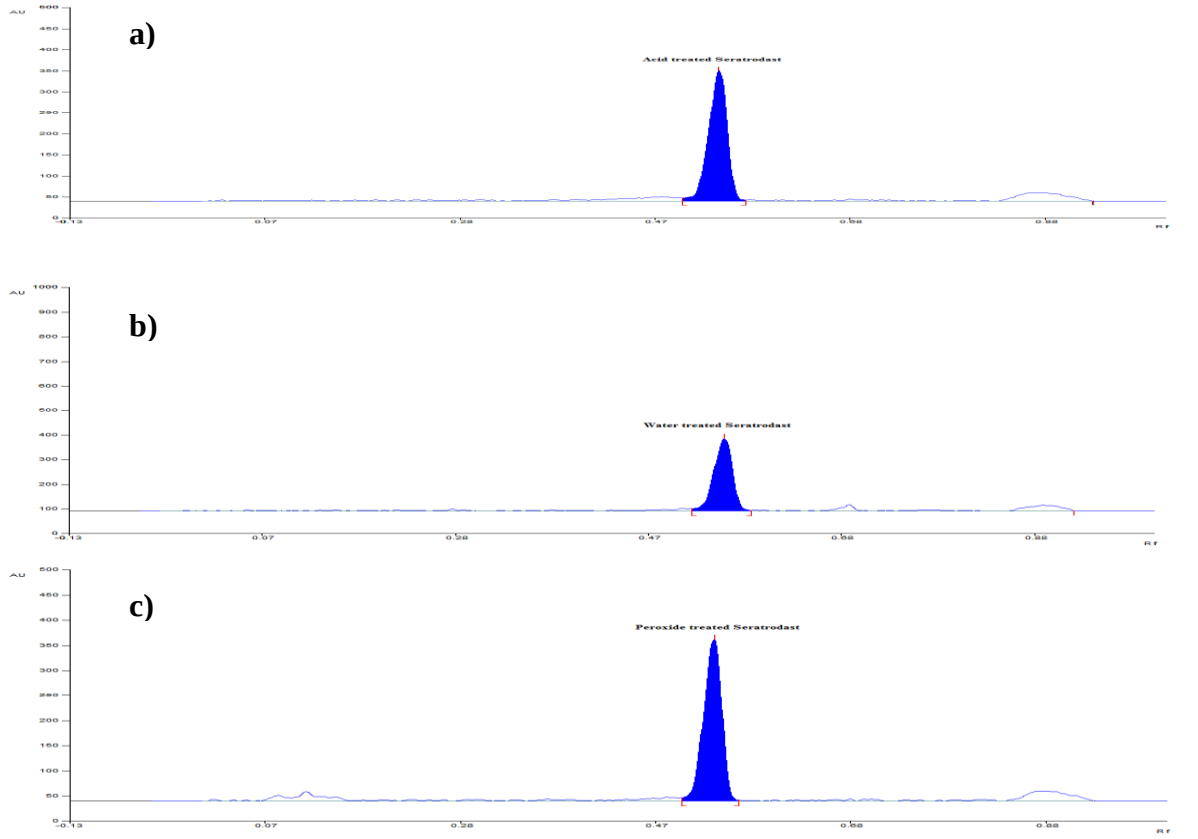


Figure 2. *Representative Densitogram of (A) base induced degradation of Seratrodast 500 ng/band (B) base induced degradation of Seratrodast 2000 ng/band showing degradant (C) Overlain spectra of Seratrodast and its alkali degradant*



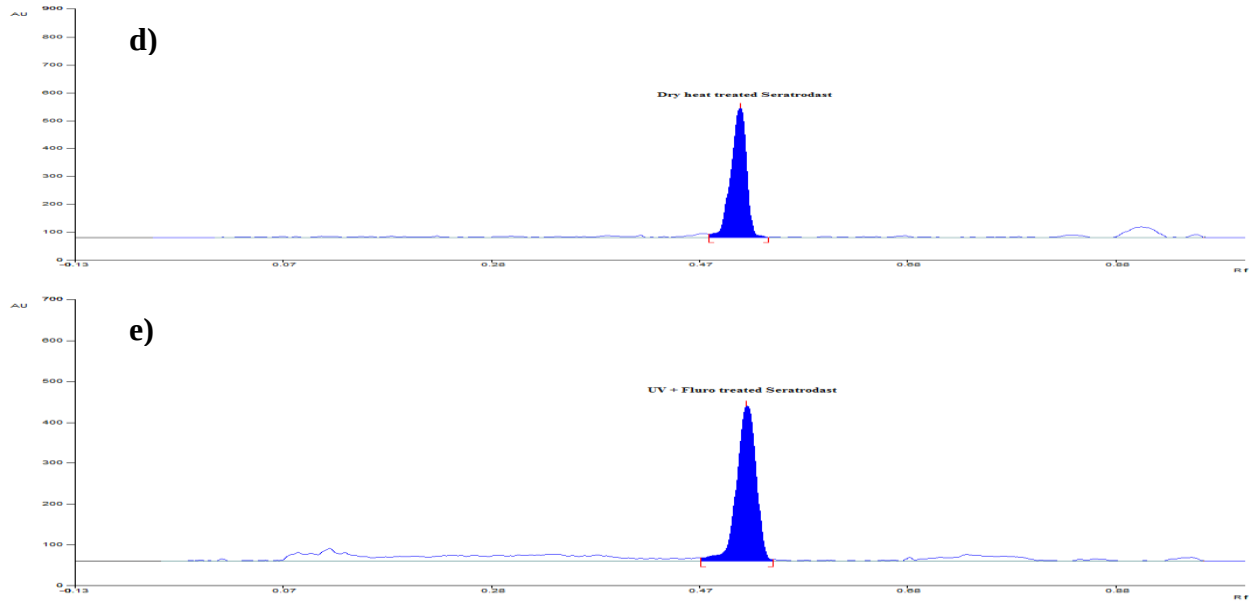


Figure 3. *Representative Densitogram of Seratrodast (500 ng/band) after a)acid degradation b) after neutral degradation c) after oxidation d) after dry heat degradation e) after photolytic degradation*

Neutral Hydrolysis

To 1 ml of 1000 µg/ml solution of Seratrodast, 1ml of distilled water was added. The above solution was kept for 4 hours at room temperature. The volume was made upto 10 ml with methanol. 5 µl of the resultant solution was then applied at TLC plate and densitogram was developed. 69.05% of Seratrodast was recovered with no peak of degradant. Representative densitogram obtained for sample subjected to neutral hydrolysis is shown in Figure 3 b.

Degradation under oxidative condition

To 1 ml of 1000 µg/ml solution of Seratrodast, 1 ml of 3% H_2O_2 was added. The above solution was kept for 1 hour at room temperature. The volume was made upto 10 ml with methanol. 5 µl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 74.81% of Seratrodast was recovered with no peak of degradant. Representative densitogram obtained for sample subjected to oxidation is shown in Figure 3 c

Degradation under dry heat

Dry heat studies were performed by keeping drug sample in oven (80^0 C) for a period of 24 hours. Sample was withdrawn, dissolved in methanol and diluted to get 100 µg/ml. 5 µl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 89.32 % Seratrodast was recovered with no peak of degradant. Representative densitogram obtained for sample subjected to dry heat is shown in Figure 3 d

Photo-degradation studies

The photo degradation study of the drug was studied by exposing the drug to UV light providing illumination of NLT 200 watt hr/m^2 followed by white fluorescence light of NLT 1.2 million Lux-Hr. After exposure accurately weighed 10 mg of drug was transferred to 10 ml of volumetric flask; the volume was made up with methanol to obtain 1000 µg/ml solution. 1 ml of the resultant solution was then diluted with methanol to get the concentration of 100 µg/ml. 5 µl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 92.75% of Seratrodast was recovered with no peak of degradant. Representative densitogram obtained for sample subjected to photolysis is shown in Figure 3 e

Validation of analytical method

Specificity

The specificity of the method was ascertained by peak purity profile studies. The peak purity values were found to be more than 0.998, indicating the no interference of any other peak of degradation product, impurity or matrix.

Linearity

From the standard stock solution (1000 µg/ml) of Seratrodast, solution was prepared containing 100 µg/ml of Seratrodast. This solution was further used for spotting. Six replicates per concentration were spotted. The linearity (relationship between peak area and concentration) was determined by analyzing six concentrations over the concentration range of 100-600 ng/band for Seratrodast .The peak areas were plotted against the corresponding concentrations to obtain the calibration curve. The results found to be linear with regression equation of y=10.47x+1614 with $R^2 = 0.994$.

Precision

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the intra-day studies 3 replicates of 3 concentrations were analyzed on the same day, and percentage RSD was calculated. For the inter day variation studies, 3 replicates of 3 concentrations were analyzed on 3 consecutive days and percentage RSD were calculated. For intraday precision and inter-day precision results obtained are shown in Table 1.

Table 1. *Intraday and interday variation studies data for Seratrodast*

Concentration (µg/ml)	Intra-day Precision			Inter-day Precision		
	Average area	% Recovery	% R.S.D	Average area	% Recovery	% R.S.D
200	3841.3	106.36	1.19	3829.4	105.79	0.43
	3752.8	102.14		3841.3	106.36	
	3814.6	105.09		3861.8	107.34	
400	5673.3	96.93	1.41	5906.6	102.49	0.76
	5778.7	99.44		5863.7	101.47	
	5832.8	100.74		5817.1	100.36	
500	6752	98.15	0.61	6899.9	100.97	1.44
	6679.7	96.76		6847.3	99.97	
	6750.2	98.11		6710.2	97.35	

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

From the linearity data the limit of detection and quantitation was calculated, using the formula LOD = 3.3 σ/ S and LOQ = 10 σ/ S where σ is standard deviation of the y intercept of linearity equation and S is slope of the calibration curve of the analyte. The LOD and LOQ were found to be 10.03 ng/ band and 30.42 ng/band respectively.

Assay

Seretra – 80 tablet formulation analysis was carried out as mentioned under section preparation of sample solution. Procedure was repeated for six times. Sample solution was applied and area was recorded. Basic concentration of sample chosen was 200 ng/band from tablet solution. Concentration and % recovery was determined from linear equation. Assay results obtained are shown in Table 2.

Table 2. *Assay of marketed formulation*

Drug	Peak Area	Amount Recovered (µg/ml)	%Recovery	± % RSD
Seratrodast	3752	204.20	102.10	1.06
	3702.8	199.50	99.75	
	3650.6	194.52	97.26	
	3695.4	198.79	99.39	
	3646.1	194.09	97.04	
	3675.1	196.86	98.43	

Accuracy

To check accuracy of the method, recovery studies were carried by spiking the standard drug to the tablet solution, at three different levels 50, 100 and 150 %. Basic concentration of sample chosen was 200 ng/band. % recovery was determined from linearity equation. The results obtained are shown in Table 3.

Table 3. *Accuracy Studies of Seratrodast*

Level	Amount of sample taken (ng/band)	Amount standard spiked (ng/band)	Area	% Recovery	± % RSD
50%	200	100	4830.8	102.41	0.68
			4789.9	101.11	
			4797.5	101.35	
100%	200	200	5806.7	100.11	0.42
			5796.1	99.85	
			5772.4	99.29	
150%	200	300	6917.6	101.31	0.29
			6891.7	100.82	
			6919.5	101.35	

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which Detection wavelength , chamber saturation time were altered, Time was also changed from spotting to development and development to scanning and the effect on the area was noted. It was found that method is robust.

3. CONCLUSION

A simple, precise, accurate, reproducible, and stability-indicating HPTLC method without interference from the excipients or from degradation products has been developed and validated for the determination of Seratrodast as bulk drug and in tablet dosage form. The developed method can be used for quantitative analysis of Seratrodast in pharmaceutical dosage form. The method was developed by using easily available and cheap solvents for analysis of drug hence can be considered as economic.

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