A NEW VALIDATED METHOD FOR THE ESTIMATION OF SPARSENTAN IN BULK AND PHARAMCEUTICAL DOSEAGE FORM BY - RPHPLC

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Abstract

The stability of innovative drug formulation and the development of appropriate stability-indicating methodologies are top priorities in contemporary pharmaceutical studies. The current work has developed and validated a thorough stability-indicating HPLC-PDA method for the determination of Sparsentan, a developing and novel endothelin and angiotensin II receptor antagonist, to reduce levels of protein in the urine (proteinuria) in patients who have a kidney disease called primary immunoglobulin A nephropathy (IgAN), The stability of Sparsentan was examined under different stress conditions. Sparsentan was found to be susceptible to Acid degradation. effective separation of Sparsentan and its induced degradation products was achieved using isocratic elution mode on Discovery C18 150 x 4.6 mm, 5µm maintained at 26.5 °C. The mobile phase used was comprised of 80.0 mm 0.1% OPA buffer (pH 2.8 ± 0.05) and acetonitrile (20 by volume), delivered at flow rate of 1.0 mL/min. The photo diode array signal for Sparsentan was monitored at 245.0 nm over a concentration range of 10–60 µg/mL, it is obvious that the recommended method is precise, dependable, economical, and time-saving. Because of this, it can be used for routine quality assurance and stability testing of Sparsentan in tablet form.

Keywords: RP-HPLC, Sparsentan, Method development, Forced Degradation

INTRODUCTION

Globally, IgA nephropathy, or IgAN, is the most prevalent glomerular disease. The treatment plan is still debatable, though. Over the past ten years, a huge number of clinical trials have been conducted to investigate the use of corticosteroids in greater detail and to introduce new therapeutic options, such as two recently authorized medications for IgAN. Sodium glucose cotransporter 2 inhibitors are showing promise as a treatment for chronic kidney illnesses caused by proteinuria, and IgAN is probably not an exception.[1,2] One of the main causes of glomerulonephritis and renal failure is immunoglobulin A (IgA) nephropathy, or IgAN. The condition is defined by the deposition of IgA in the glomerular mesangium.[3,4] Immune-mediated injury to the basement

membrane causes proteinuria, hematuria, and insufficient renal function. Important findings have resulted from recent international joint efforts that have considerably expanded our understanding of the immunopathogenesis of IgAN, Multicenter networks have also made it easier to plan and conduct clinical trials holistically, which has provided important information about immunotherapy for IgAN. IgAN is caused by aberrant immune responses, which lead to IgA deposits in the glomerulus, increased podocyte permeability, and interstitial fibrosis.[5,6] It is important to remember that although infectious diseases frequently precede IgAN by causing a dysregulated immune response, IgAN does not have an infectious etiology.[7,8] There is no proof that IgAN is caused by any infectious pathogen. Rather, a variety of clinical and subclinical stimuli, as well as genetic variables connected to IgA glycosylation, seem to stimulate the immune system. IgAN is the most frequent cause of glomerulonephritis, but because many cases are asymptomatic and require a kidney biopsy for a clear diagnosis, the exact prevalence of the condition is still unknown.[9]

Background of analyte: - It is a dual antagonist of the endothelin type A receptor (ETAR) and the angiotensin II (Ang II) type 1 receptor (AT1R), and it is prescribed to patients with primary immunoglobulin A nephropathy who have the potential to progress rapidly. The FDA approved it under accelerated approval based on the reduction of proteinuria in adults with primary immunoglobulin A nephropathy (IgAN) who are at risk of rapid disease progression. It is a chemical that functions as a dual antagonist of the angiotensin II (Ang II) type 1 receptor (AT1R) and the endothelin type A receptor (ETAR).[10] It specifically inhibits the actions of two strong vasoconstrictor and mitogenic agents, Ang II and endothelin 1 (ET-1), at their respective receptors and has two clinically proven modes of action. Immunoglobulin A Nephropathy (IgAN) is a pathological condition marked by elevated production of galactose-deficient IgA1 (Gd-IgA1) antibodies. ET-1 and Ang II are involved in this process. Mesangial cell activation and proliferation are triggered by Gd-IgA1 antibodies and are in turn stimulated by the synthesis of Ang II and ET-1. structure elucidation was figured out in Figure No-1.
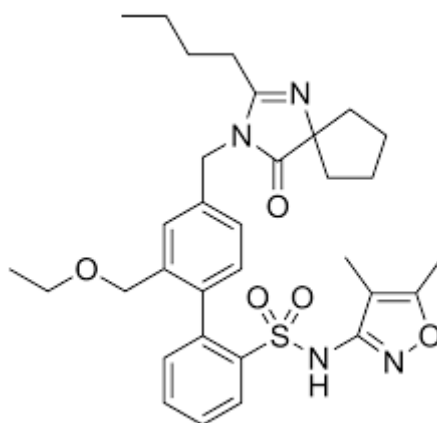

Figure No-1 Structure of Sparsentan

The chemical stability of novel drug molecules is crucial for the safety and effectiveness of the drug product; it helps choose the right formulation and packaging and offers ideal shelf life and storage conditions. Drug products are forced to degrade under extreme conditions to find possible degradation products.[13] This process also aids in establishing degradation routes and validating stability-indicating techniques. Upon literature survey, few methods were reported for determination of Sparsentan in biological fluids and/or pharmaceutical formulations using different analytical techniques; UV–VIS spectroscopy methods, and chromatographic

method, Although, few HPLC methods were previously reported but up to our knowledge no stability-indicating HPLC method has been published yet for determination of Sparsentan.[14,15] Accordingly, the proposed work aims to develop and validate for the first time a stability-indicating HPLC-PDA for determination of Sparsentan in its pharmaceutical dosage form. The chemical stability of Sparsentan was evaluated under different stress conditions such as hydrolysis, oxidation, photolytic, and thermal stress conditions.

Chemicals and Equipment's: -

Pure medication of Sparsentan were delivered by MSN(Hyderabad), india with 99.98%. An India Mart pharmacy provided Sparsentan tablet from Biochemix Healthcare Private Limited Himachal Pradesh. All the chemicals and buffers used in this method were given by Rankem in India, Analysis was performed on waters alliance HPLC system (Waters, USA) equipped with quaternary pump (M00SM4493M), autosampler (M17VSM029N), column oven (B18HLP981G) and photodiode array detector (2998PDA).

Buffer preparation: -
0.1%OPA Buffer: 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water(pH-2.8)
Standard solutions: Accurately weighed 20mg of Sparsentan is transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (400µg/ml of Sparsentan), from the stock solution, 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (40µg/ml of Sparsentan).

Analysis of Sparsentan in its pharmaceutical formulation: -
10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (2000µg/ml of Sparsentan), from the stock solution 0.2ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (40µg/ml of Sparsentan)

Optimized Conditions: -
Chromotography method was achieved using isocratic elution mode on Discovery C18 150 x 4.6 mm, 5▯m stationary phase, The mobile phase used was comprised of 80.0 mm 0.1% OPA buffer (pH 2.8 ± 0.05) and acetonitrile (20 by volume), delivered at flow rate of 1.0 mL/min, The column temperature was kept at 26.5 °C. for each sample, 10 µL was injected in hplc. The photo diode array signal for Sparsentan was monitored at 245.0 nm, WATERS HPLC, model: 2695 SYSTEM with Photo diode array detector was used for the development and method validation, with an automated sample injector with software Empower 2, Fig no 2
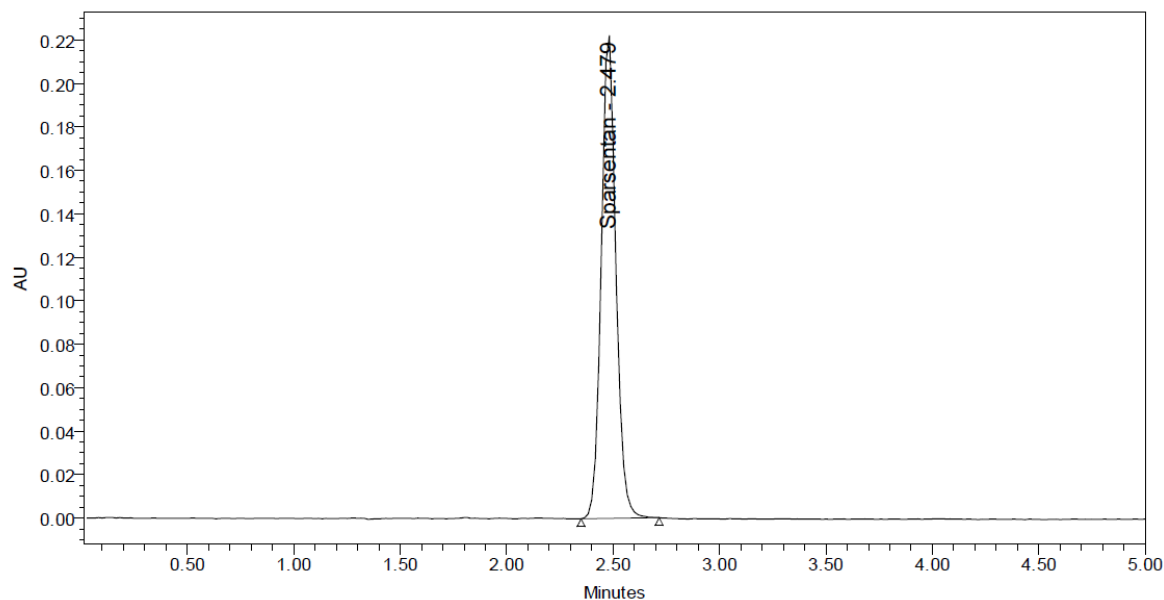
	Peak Name	RT	Area	% Area	Height	USP Plate Count	USP Tailing
1	Sparsentan	2.479	1162330	100.00	219422	6236.3	1.1

Figure No-2 Optimized Chromotogram

METHOD VALIDATION

The validation of the HPLC method was carried out in accordance with the ICH recommendations for the simultaneous estimation of Sparsentan material to show that the method is suitable for routine analysis.

System suitability:

The system suitability parameters were determined by preparing standard solutions of Sparsentan (40ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined, Results are displayed in Table 1, Figure No. 3.

The % RSD for the area of six standard injections results should not be more than 2%.

Specificity (Selectivity): Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific. Figure No. 4 and 5.

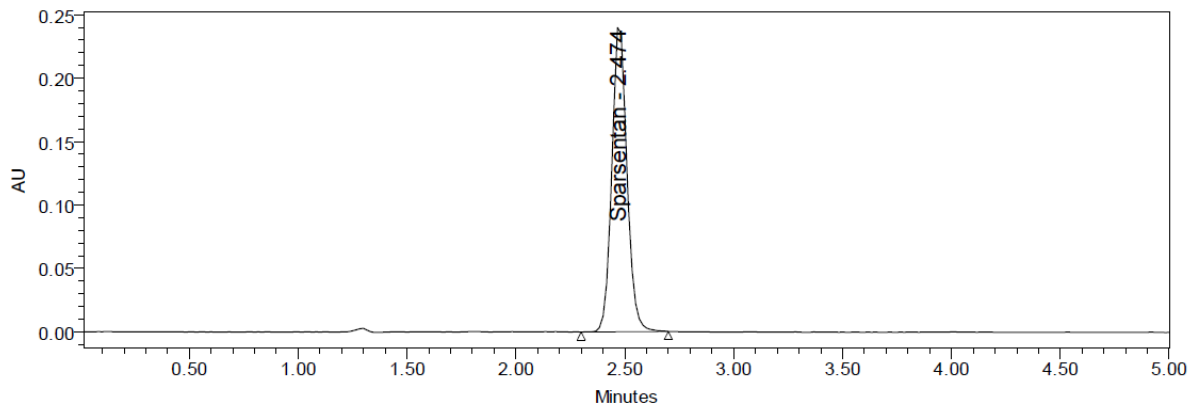| S no	Sparsentan		
Injects	**RT(min)**	**USP Plate Count**	**Tailing**
1	2.474	6228	1.10
2	2.479	6237	1.06
3	2.483	6317	1.07
4	2.485	6206	1.08
5	2.487	6274	1.09
6	2.496	6322	1.06

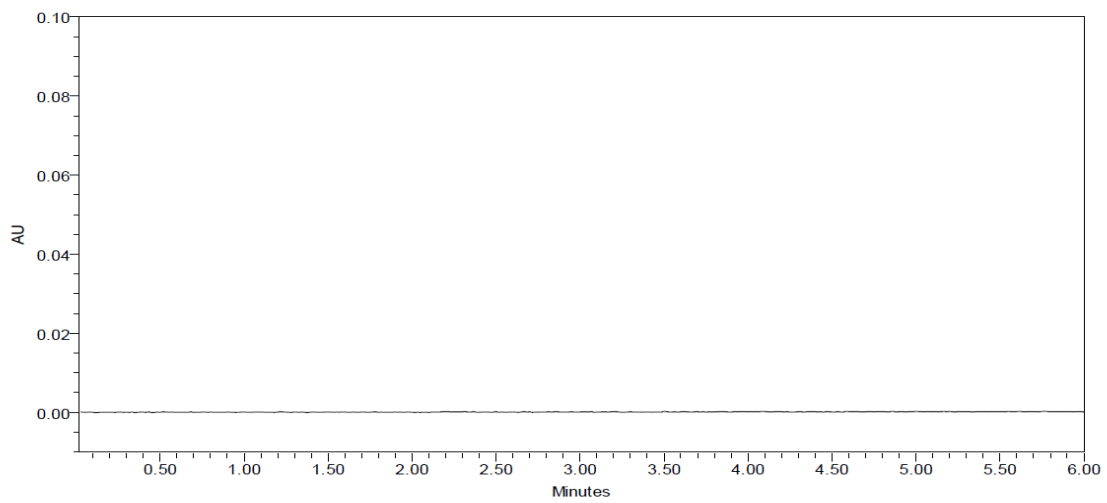**Figure No-3: System suitability Chromatogram of Sparsentan**
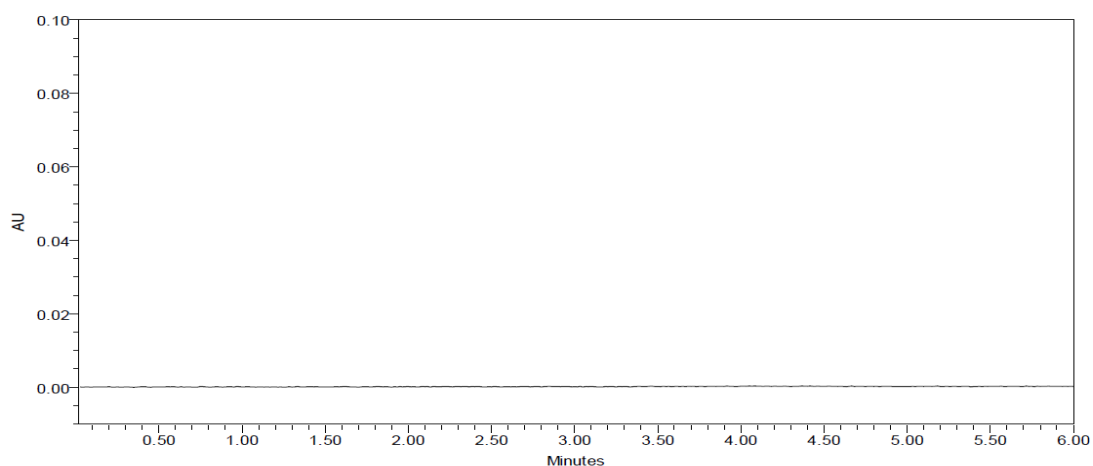

Figure No. 4 Blank
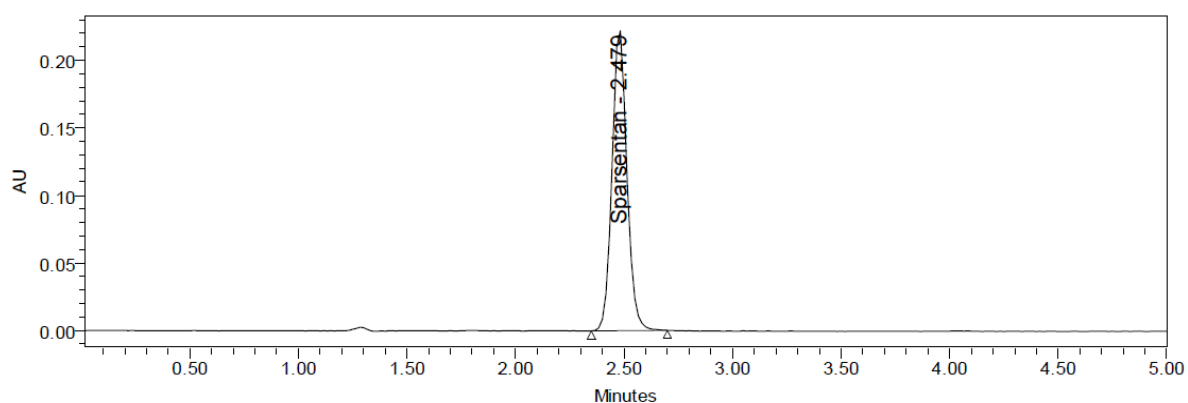
Figure No. 5 Placebo

Figure No. 6 Chromatograms of Specificity

Discussion: Sparsentan had retention durations of 2.479 minutes. Using this strategy, we were unable to discover any interfering peaks in the blank and placebo at the retention times of these medications. It was said that this approach was specific.

Linearity and construction of calibration curve

Sparsentan linearity was examined across a concentration range of 10–60 μg/mL. Using the previously mentioned chromatographic settings, triplicate injections of the prepared solutions were made into the HPLC-PDA system. Peak area values were plotted against the appropriate concentrations of Sparsentan to create the calibration graph was plotted in Figure No. 5, and the regression equation was then calculated. Results are displayed in Table 2.

Table 2: Sparsentan Linearity

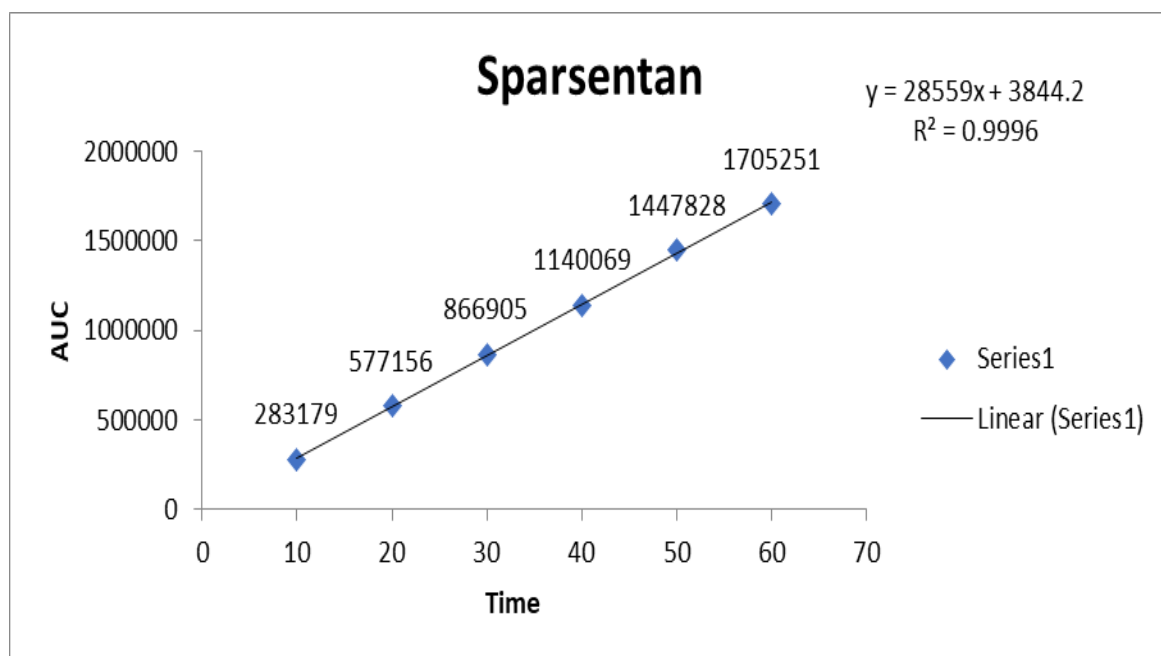| Linearity Level (%)	Concentration (ppm)	Area
0	0	0
25	10	283179
50	20	577156
75	30	866905
100	40	1140069
125	50	1447828
150	60	1705251

Figure No. 7: Sparsentan Calibration curve

Discussion: Accuracy is established across the specified range of the analytical procedure. Three concentration levels, in triplicates, (50.0, 100.0 and 150.0 µg/mL) of pure samples of Sparsentan were analyzed by the proposed method.

Table 3: Accuracy (%Recovery data)

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	20	19.90	99.50	
	20	19.84	99.18	
	20	19.84	99.20	
100%	40	40.25	100.62	
	40	40.09	100.24	**100.11%**
	40	39.94	99.85	
150%	60	60.89	101.49	
	60	60.40	100.67	
	60	60.13	100.22	

Observation: - The standard addition method was used to prepare three levels of accuracy samples. For every accuracy level, three injections were administered, and the mean percentage of recovery for Sparsentan was found to be 100.11%.

System Precision: The system precision was performed by analyzing six replicate injections of standard solution at 100% of the specified limit with respect to the working strength of Sparsentan. Results of peak area are summarized in Table 4.

Table :4 System precision data

S. No	Area of Sparsentan
1.	1165196
2.	1162011
3.	1144366
4.	1172628
5.	1152221
6.	1130856
Mean	**1154546**
S.D	**15273.3**
%RSD	**1.3**

Discussions: The % RSD for the peak areas of Sparsentan obtained from six replicate injections of standard solution was within the limit.

Method Precision: Six working sample solutions are injected and the % Amount found was calculated and %RSD was found to be 0.5.

Table 5: **Method precision data**

Injection	Sparsentan
1.	1152652
2.	1151474
3.	1161906
4.	1154036
5.	1160264
6.	1152232
Mean	**1155427**
S.D	**4491.0**
%RSD	**0.4**

Discussions: From the above results, the % RSD of method precision study was within the limit for Sparsentan.

Robustness parameter

Samples were injected in triplicate under robustness settings that included Flow minus (0.9 ml/min), Flow plus (1.1 ml/min), Mobile Phase minus (65:35A), Mobile Phase plus (75B:25A), Temperature minus (27°C), and Temperature plus (33°C). All the system suitability parameters passed with little to no impact. %RSD was not over the upper bound.

Table 6: Robustness results

Chromatographic condition	Sparsentan (SRN)
Flow rate (-) 0.9ml/min	0.7
Flow rate (+) 1.1ml/min	0.6
Mobile phase (-) 45B:55A	1.4
Mobile phase (+) 55B:45A	0.4
Temperature (-) 25°C	0.6
Temperature (+) 35°C	1

Stress stability studies

Stress studies were carried out as per ICH guidelines under different acid, base, oxidative, photolytic, and thermal conditions.[15]

Table 7: Forced degradation conditions for Sparsentan.

S.NO	Degradation Condition	% Drug UnDegraded	% Drug Degraded
1	Acid	91.98	8.02
2	Alkali	96.56	3.44
3	Oxidation	96.31	3.69
4	Thermal	96.94	3.06
5	UV	98.20	1.80
6	Water	99.16	0.84

Discussion: From the results, no degradation was observed when the samples were exposed to base, hydrolysis, thermal, light and water. According to the stress study, none of the degradant co-eluted with the active drug peaks formed.
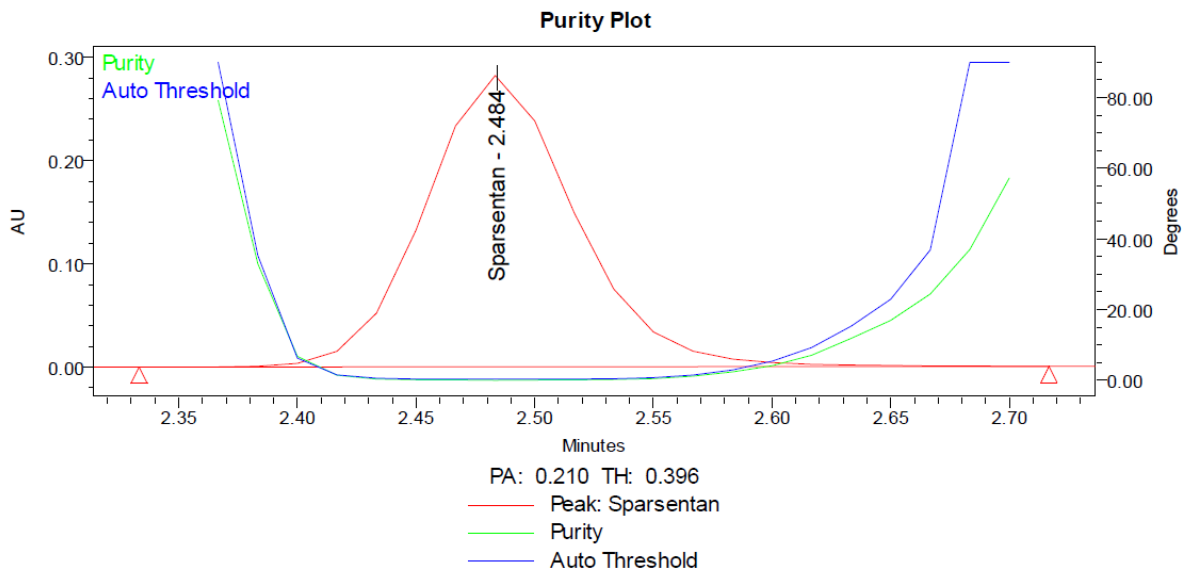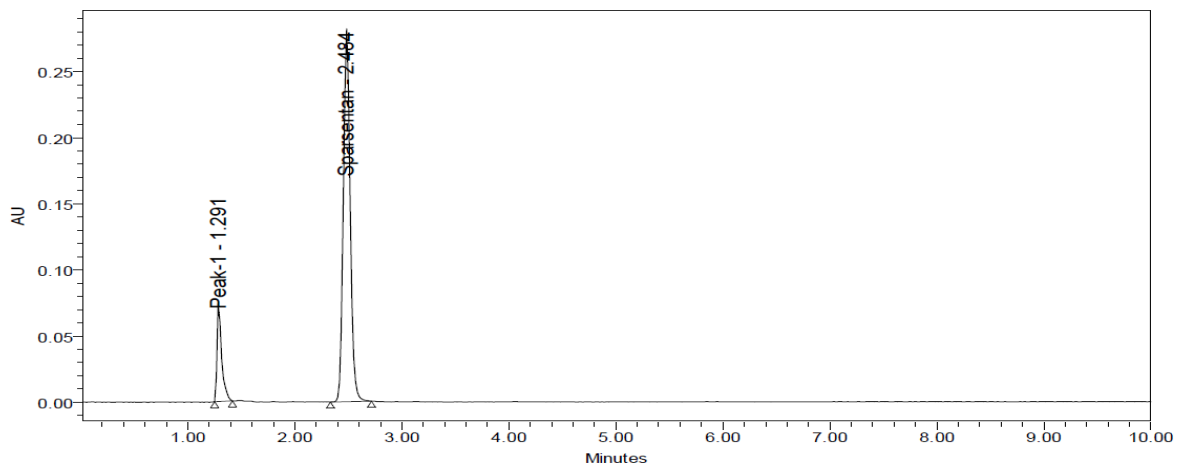

Figure No.8. Degradation purity plots

Figure No.9. Acid degradation chromatogram

Analysis of Sparsentan in its pharmaceutical formulation.

Ten tablets (each tablet labelled to contain 200 mg Sparsentan) were separately weighed, grinded and finely powdered. An amount equivalent to average weight of one tablet was accurately transferred into 100-mL volumetric flask and dissolved in 60 mL Acetonitrile and water, sonicated for 10 min. The volume was then completed to mark with Acetonitrile and water and filtered through filters of 0.22 µm pore size (Millipore, Milford, MA). Appropriate dilutions were made with deionized water. The constructed calibration graph was used to estimate the concentration of Sparsentan in tablets via the corresponding regression equation. For standard addition technique, different known concentrations of pure Sparsentan standard were added to known concentrations of the pharmaceutical dosage form and processed as described before. The recovered concentration of added Sparsentan was obtained from corresponding regression equation and then the mean percentage recovery and the standard deviation were calculated.

Table 8: Assay data of Sparsentan and % assay

S.no	Standard Area	Sample area	% Assay
1	1165196	1152652	99.44
2	1162011	1151474	99.33
3	1144366	1161906	100.23
4	1172628	1154036	99.56
5	1152221	1160264	100.09
6	1130856	1152232	99.40
Avg	1154546	1155427	99.68
Stdev	15273.3	4491.0	0.39
%RSD	1.3	0.4	0.4

Observation: - Filspari with the label "200 mg of Sparsentan" The formulation was used for the assay 99.73% of the assay for Sparsentan was obtained on average.

Conclusion

A rapid, accurate and specific HPLC-PDA method was established to determine Sparsentan in presence of its forced degradation products for the first time. A comprehensive stress stability study revealed the susceptibility of Sparsentan to acid degradation. The suggested HPLC-PDA method makes a great use of PDA in determining peak purity and choosing the appropriate wavelength for analysis. Successful assay of Sparsentan in marketed pharmaceutical formulation without interference from common tablets' excipients or possible degradation products reveals method's suitability as stability-indicating method in routine quality control laboratories.

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