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R E S E A R C H A R T I C L E

Stability Indicating Method Development and Validation for Simulatenous Estimation of Zopolrestat and Pregabalin by Using RP-HPLC

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A B S T R A C T

In this study, the active pharmaceutical components of Zopolrestat and Pregabalin in their bulk dosage forms were to be measured using a unique, straightforward, responsive, and stable RP-HPLC method that was being developed and gradually validated. For the quantitative determination of Zopolrestat and Pregabalin, a straightforward, specific, verified, and well-defined stability that exhibits gradient RP-HPLC approach has been developed. The Agilent C18 (4.6 x 150mm, 5 m) was used for the chromatographic method, which involved isocratic elution with a mobile phase made up of orthophosphoric acid (0.1%) and acetonitrile (40:60% v/v). The instrument parameters called for a flow rate of 1 ml/min and a detection wavelength of 241 nm using the UV detector. The chromatographic method was expedited using the impurity-spiked solution. The proposed method's validity was examined in accordance with the international conference on harmonization (ICH) guidelines. In relation to test concentration, LOD and LOQ were determined for the two active components and their contaminants. The plotted calibration charts had linear regression coefficients of 0.999, indicating that their linearity was within acceptable bounds. As part of the technique validation, recovery, specificity, linearity, accuracy, robustness, and ruggedness were assessed, and the findings were found to be within the acceptable range. The suggested approach is quick, easy, practical, and reasonably priced. It can be used for routine manufacturing sample analysis during stability tests and to confirm the calibre of medication samples during stability studies.

Keywords: Zopolrestat, Pregabalin, Method development, RP-HPLC, Validation

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Zopolrestat is chemically 3,4-dihydro-4-oxo-3-[[5-(trifluoromethyl)-2-benzothiazolyl] methyl]-1-phthalazineacetic acid**.** Zopolrestat is a novel potent aldose reductase inhibitors developed by Pfizer for the treatment of diabetic complications, is a potent competition inhibitor of human glyoxalase I (GLOI) in vitro. Crystal structures of GLOI in complex with zopolrestat and indomethacin, a nonsteroidal anti-inflammatory drug and moderate inhibitor of GLOI, provide a structural basis for the development of novel GLOI inhibitors with excellent pharmacokinetics profiles. Pregabalin is chemically (S)-3-(aminomethyl)-5-methylhexanoic acid. It is an anticonvulsant drug used to treat neuropathic pain conditions and fibromyalgia, and for the treatment of partial onset seizures in combination with other anticonvulsants.[1-4] Pregabalin is structurally similar to gamma-aminobutyric acid (GABA) - an inhibitory neurotransmitter. It may be used to manage neuropathic pain, postherpetic neuralgia, and fibromyalgia among other conditions. Although as per the FDA Label the mechanism of action has not been definitively defined, there is evidence that Pregabalin exerts its effects by binding to the α2δ subunit of voltage-dependent calcium channels. Pregabalin is marketed by Pfizer under the trade name Lyrica and Lyrica CR (extended release). It may have dependence liability if misused but the risk appears to be highest in patients with current or past substance use disorders.[5-9]

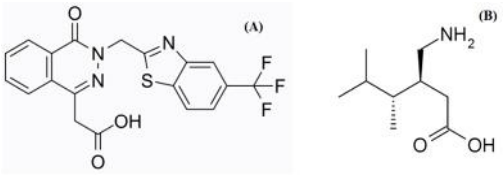

Fig. 1: Structure of Zopolrestat (A) and Pregabalin (B)

2. Materials and Methods

Zopolrestat and Pregabalin pure drugs (API) procured from Yarrow chem products, Mumbai. Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen orthophosphate buffer, Ortho- phosphoric acid. All the above chemicals and solvents are from Rankem Ltd.
Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50.
Preparation of Standard stock solutions: Accurately weighed 75 mg of Zopolrestat, 75 mg of Pregabalin and transferred to 50ml & 50ml volumetric flasks. 3/4 th of diluents was added and solicited for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1 and 2. (1500µg/ml ZOPOL of and 1500µg/ml of PREGA).

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (150 µg/ml of ZOPOL and 150µg/ml of PREGA). Preparation of Sample stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml 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. (1500 µg/ml of ZOPOL and 1500 µg/ml of PREGA).

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (150µg/ml of ZOPOL and 150µg/ml of PREGA).

Preparation of buffers

0.01N KH2PO4 Buffer: Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 5.4 with dil. Orthophosphoric acid solution. 0.1%OPA Buffer: 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

Validation

System suitability parameters

The system suitability parameters were determined by preparing standard solutions of Zopolrestat (150ppm) and Pregabalin (150ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results should not be more than 2%.

Specificity: 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.

Precision

Preparation of Standard stock solutions: Accurately weighed 75 mg of Zolpolrestat, 75 mg of Pregabalin and transferred to 50ml&50ml volumetric flasks. 3/4 th of diluents was added and solicited for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1 and 2. (1500µg/ml ZOPOL of and 1500µg/ml of PREGA)

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Linearity

Preparation of Standard stock solutions: Accurately weighed 75 mg of Zopolrestat, 75 mg of Pregabalin and transferred to 50ml&50ml volumetric flasks. 3/4th of diluents was added and solicited for 10 minutes. Flasks

were made up with diluents and labeled as Standard stock solution 1 and 2. (1500µg/ml ZOPOL of and 1500µg/ml of PREGA)

25% Standard solution: 0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (37.5µg/ml of ZOPOL, and 37.5 µg/ml of PREGA)

50% Standard solution: 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (75µg/ml of ZOPOL, and 75 µg/ml of PREGA)

75% Standard solution: 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (112.5µg/ml of ZOPOL, and 112.5µg/ml of PREGA)

100% Standard solution: 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (150µg/ml of ZOPOL, and 150µg/ml of PREGA)

125% Standard solution: 1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (187.5µg/ml of ZOPOL and 187.5µg/ml of PREGA)

150% Standard solution: 1.5ml each from two standard stock solutions was pipette out and made up to 10ml. (225µg/ml of ZOPOL and 225µg/ml of PREGA)

Accuracy

Preparation of Standard stock solutions: Accurately weighed 75 mg of Zopolrestat, 75 mg of Pregabalin and transferred to 50ml&50ml volumetric flasks. 3/4th of diluents was added and solicited for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1 and 2. (1500µg/ml ZOPOL of and 1500µg/ml of PREGA).

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent. Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Degradation Studies17-24

Oxidation: To 1 ml of stock solution of Zopolrestat and Pregabalin, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 600C. For HPLC study, the resultant solution was diluted to obtain 150µg/ml & 150µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies: To 1 ml of stock s solution Zopolrestat and Pregabalin, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 600C. The resultant solution was diluted to obtain 150µg/ml & 150µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:

To 1 ml of stock solution Zopolrestat and Pregabalin, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 600C. The resultant solution was diluted to obtain 150µg/ml & 150µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies:

The standard drug solution was placed in oven at 105°C for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 150µg/ml & 150µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies:

The photochemical stability of the drug was also studied by exposing the 150µg/ml & 150µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 150µg/ml & 150µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies:

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to 150µg/ml & 150µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

3. Results and Discussion

System suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines. According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.[25]

Table 1: System suitability parameters for Zopolrestat and Pregabalin.

S No	Pregabalin			Zopolrestat			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.149	3226	1.15	2.901	3845	1.08	4.4
2	2.154	3380	1.18	2.915	3846	1.08	4.3

3	2.159	3315	1.21	2.91	3832	1.1	4.3
4	2.166	3292	1.19	2.916	3946	1.1	4.4
5	2.169	3314	1.18	2.92	3981	1.08	4.4
6	2.179	2515	1.29	2.93	3054	1.22	3.7

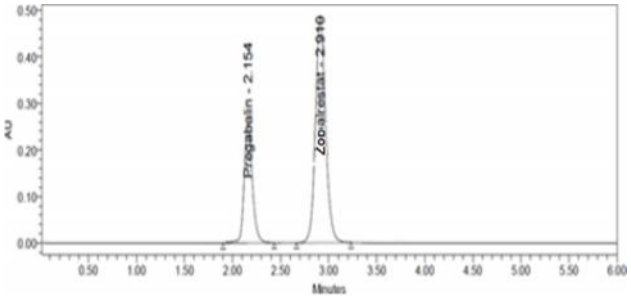

Fig 2: System suitability Chromatogram

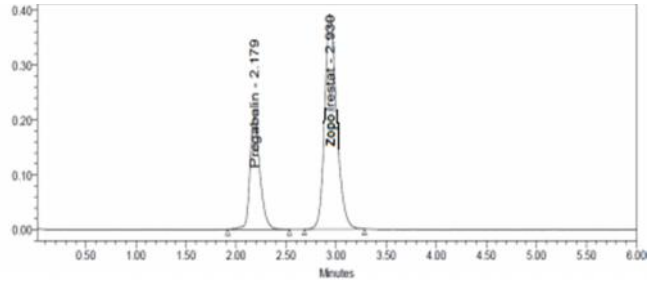

Fig 3: Optimized Chromatogram of Zopolrestat and Pregabalin

Validation

Linearity

Six linear concentrations of Zopolrestat (37.5- 225µg/ml) and Pregabalin (18.75-112.5µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Zopolrestat was y =20545.x + 16173 and of Pregabalin was y = 18476x + 10803 Correlation coefficient obtained was 0.999 for the two drugs.

Table 2: Linearity table for Zopolrestat and Pregabalin.

Zopolrestat		Pregabalin	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
37.5	793975	18.75	392023
75	1538713	37.5	696010
112.5	2335643	56.25	1033486
150	3160302	75	1385625
187.5	3852671	93.75	1736003
225	4611087	112.5	2107476

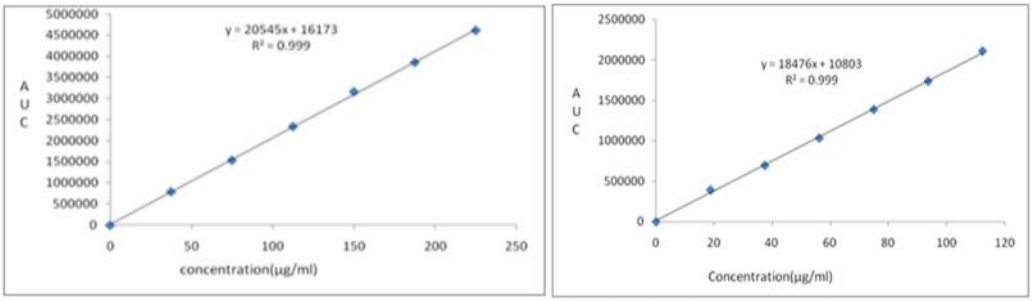

Fig 13: Calibration curve of Zopolrestat and Pregabalin.

Table 3: Repeatability table of Zopolrestat and Pregabalin.

S. No	Area of Zopolrestat	Area of Pregabalin
1	3115267	1310152
2	3145891	1311046
3	3152013	1310287
4	3140181	1312589
5	3142258	1309030
6	3140106	1304812

Mean	3139286	1309653
S.D	12592.2	2647.1
%RSD	0.4	0.2

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

Table 4: Accuracy table of Zopolrestat

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	75	74.08	98.77	98.98%
	75	74.39	99.18	
	75	74.62	99.49	
100%	150	150.49	100.33	
	150	147.25	98.17	
	150	148.32	98.88	
150%	225	223.3	99.25	
	225	221.78	98.57	
	225	220.86	98.16	

Table 5: Accuracy table of Pregabalin

% Level		Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	37.5	37.04	98.54	99.35%
	37.5	37.54	100.1	
	37.5	37.34	99.56	
100%	75	73.67	98.22	
	75	74.06	98.74	
	75	75.16	100.22	
150%	112.5	111.51	99.12	
	112.5	112.15	99.69	
	112.5	112.23	99.76	

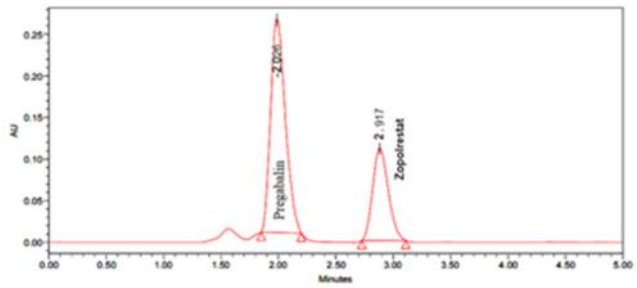

Fig 4: Repeatability Chromatogram

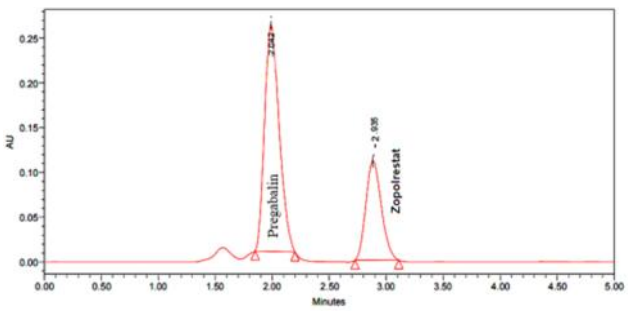

Fig 5: Chromatogram of Accuracy 50%

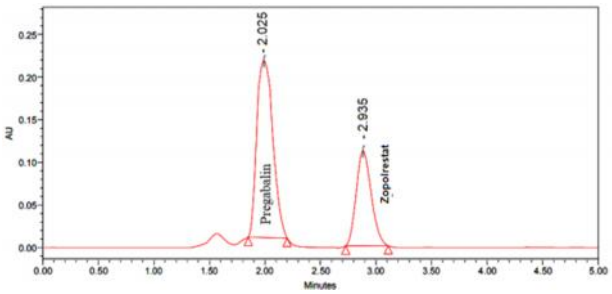

Fig 6: Chromatogram of Accuracy 100%

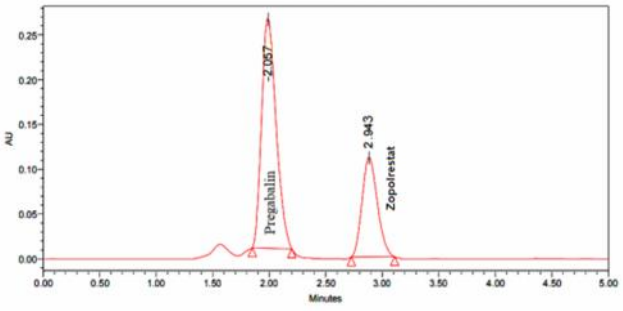

Fig 7: Chromatogram of Accuracy 150%

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %recovery was obtained as 98.98% and 99.35% for Zopolrestat and Pregabalin respectively.[26]

Table 6: Sensitivity table of Zopolrestat and Pregabalin.

Molecule	LOD	LOQ
Zopolrestat	0.2	0.62
Pregabalin	0.18	0.56

Table 7: Robustness data for Zopolrestat and Pregabalin.

S. No.	Condition	%RSD of Zopolrestat	%RSD of Pregabalin
1	Flow rate (-) 0.7ml/min	1.3	1.3
2	Flow rate (+) 0.9ml/min	1.3	1.3
3	Mobile phase (-) 50B:50A	0.4	0.2
4	Mobile phase (+) 40B:60A	0.7	0.8
5	Temperature (-) 25°C	1.8	1.8
6	Temperature (+) 35°C	0.3	0.6

Assay: Standard preparations are made from the API and sample preparations are from formulation. Both sample and standards are injected six homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference. The average % assay was calculated and found to be 99.73% and 99.32% for Zopolrestat and Pregabalin respectively.[27-29]

Table 8: Assay of Zopolrestat and Pregabalin

	Zopolrestat			Pregabalin		
S. No.	**Standard Area**	**Sample area**	**% Assay**	**Standard Area**	**Sample area**	**% Assay**
1	3105736	3115267	98.96	1300458	1310152	99.36
2	3146003	3145891	99.94	1316596	1311046	99.42
3	3145407	3152013	100.13	1320473	1310287	99.37
4	3159858	3140181	99.76	1314421	1312589	99.54
5	3146567	3142258	99.82	1321248	1309030	99.27
6	3145825	3140106	99.75	1322787	1304812	98.95
Avg	3141566	3139286	99.73	1315997	1309653	99.32
SD	18417.3	12592.2	0.4	8222.1	2647.1	0.2
%RSD	0.6	0.4	0.4	0.6	0.2	0.2

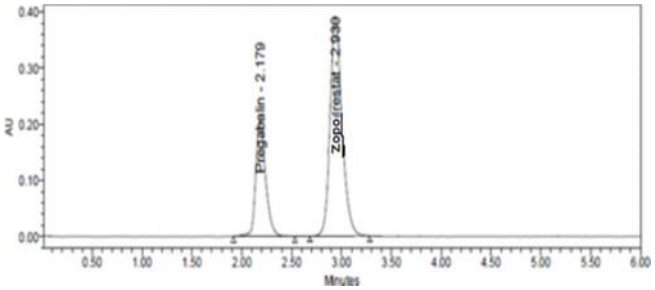

Fig. 8: Chromatogram of working standard solution.

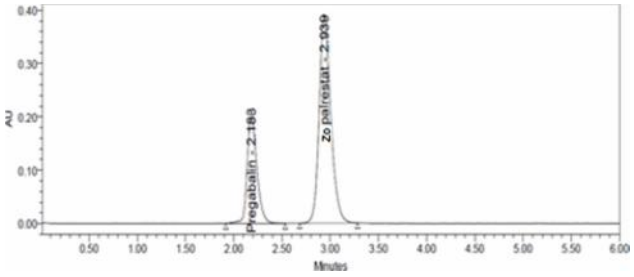

Fig. 9: Chromatogram of working sample solution.

Degradation Studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

Table 9: Degradation Data of Zopolrestat and Pregabalin

S. No.	Degradation Condition	Zopolrestat			Pregabalin		
		% Drug Degraded	Purity Angle	Purity Threshold	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.46	0.155	0.373	4.58	0.605	1.474
2	Alkali	2.88	0.14	0.366	2.48	0.988	1.445
3	Oxidation	1.44	0.105	0.374	1.52	0.172	0.976
4	Thermal	0.78	0.12	0.367	0.81	0.158	0.415
5	UV	0.72	0.128	0.361	0.55	0.132	0.454
6	Water	0.74	0.143	0.371	0.49	0.346	0.504

Table 10: Summery

Parameters		Zopolrestat	Pregabalin	Limit
Linearity Range (µg/ml)		37.5-225µg/ml	18.75-112.5µg/ml	R< 1
Regression coefficient		0.999	0.999	
Slope (m)		20545	18476	
Intercept(c)		16173	10803	
Regression equation (y=mx+c)		y = 20545x + 16173	y = 18476x + 10803	
Assay (% mean assay)		99.73%	99.32%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		0.6	0.6	NMT 2.0%
Method precision %RSD		0.4	0.2	NMT 2.0%
Accuracy % recovery		98.98%	99.32%	98-102%
LOD		0.2	0.18	NMT 3
LOQ		0.62	0.56	NMT 10
Robustness	FM	1.3	1.3	%RSD NMT 2.0
	FP	1.3	1.3	
	MM	0.4	0.2	
	MP	0.7	0.8	
	TM	1.8	1.8	
	TP	0.3	0.6	

4. Conclusion

A simple, Accurate, precise method was developed for the simultaneous estimation of the Zopolrestat and Pregabalin in dosage form. Retention time of Zopolrestat and Pregabalin were found to be 2.930 min and 2.179 min. %RSD of the Zopolrestat and Pregabalin were and found to be 0.4and 0.2 respectively. %Recovery was obtained as 98.98% and 99.32% for Zopolrestat and Pregabalin respectively. LOD, LOQ values obtained from regression equations of Zopolrestat and Pregabalin were 0.02, 0.06 and 0.26, 0.77 respectively. Regression equation of Zopolrestat is y=20545x+16173, and y= 18476x + 10803 of Pregabalin. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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