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Research Article

METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF EPALRESTAT AND PREGABALIN IN HUMAN PLASMA BY USING RP-HPLC

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ABSTRACT

A simple, rapid, sensitive, precise and accurate high-performance liquid chromatography method was developed for simultaneous determination of Epalrestat and Pregabalinin human plasma using Glipizide as an internal standard (ISTD). The analytes were extracted from 500 µL aliquots of a human plasma sample by direct protein precipitation technique using acetonitrile. Evaluation of content of the drugs was done by employing a mixture of acetonitrile and 0.1% orthophosphoric acid (OPA) buffer in the ratio of 45:55 v/v as the mobile phase with a flow rate of 1 mL/min and injection volume of 10µL. Chromatographic separation was accomplished using Symmetry C18 150 X 4.6mm, 5µm analytical column and the effluents were monitored at 220 nm with a photodiode array (PDA) detector. The total run time was 8 min with a retention time of Epalrestat, PregabalinandGlipizide was 3.828, 4.699, and 2.463 min respectively. Linearity was established at a concentration range of 0.250-5.00 µg/mL for Epalrestat and 0.160-3.25 µg/mL for Pregabalin. The method was validated as per the US-FDA guidelines and the results were within the acceptance criteria and proposed method was successfully applied for the simultaneous determination of Epalrestat and Pregabalinin human plasma.

Keywords: Epalrestat, Pregabalin, Protein Precipitation, Human Plasma, RP-HPLC.

INTRODUCTION

Epalrestat is an aldose reductase inhibitor. It is chemically designated as 2-[(5*Z*)-5-[(*E*)-2-methyl-3-phenylprop-2-enylidene]-4-oxo-2-sulfanylidene-1,3-thiazolidin-3-yl] acetic acid. The chemical Formula of Epalrestat $C_{15}H_{13}NO_3S_2$. Aldose reductase reduces glucose to sorbitol. Epalrestat restrained high glucose-intervened neutrophils. Endothelial cell grip and articulation of endothelial bond particles not just through the hindrance of a PKC-subordinate pathway, yet additionally through expanded endothelial NO generation.

Epalrestat is a carboxylic corrosive subsidiary and a non-competitive and reversible utilized for the treatment of which is a standout amongst the most widely recognized long haul intricacies in patients with. It lessens the aggregation of intracellular sorbitol which is accepted to be the reason for diabetic neuropathy, retinopathy and, nephropathy. Artificially, Epalrestat is strange in that it is a medication that contains a gathering. Epalrestat is the main ARI economically accessible. It is effortlessly assimilated into the neural tissue and hinders the compound with the least symptoms.[1]

Pregabalin is an anticonvulsant tranquilizes utilized for neuropathic torment, as an aide treatment for incomplete seizures, and in summed up tension issues. It was outlined as a stronger successor to gabapentin. Pregabalin is promoted by Pfizer under the exchange name Lyrica. It is considered to have a reliance risk if abused and is named a Schedule V sedate in the U.S.It is chemically designated as (3S)-3-(aminomethyl)-5-methylhexanoic acid. The chemical Formula of Pregabalin is $C_8H_{17}NO_2$.

Pregabalin is utilized for the administration of neuropathic torment related to diabetic fringe neuropathy or spinal rope damage, and postherpetic neuralgia. It is likewise utilized as an adjunctive treatment for grown-up patients with halfway beginning seizures and administration of fibromyalgia.[2]

Glipizide is an oral hypoglycemic agent in the second-generation sulfonylurea drug class that is used to control blood sugar levels in patients with type 2 diabetes mellitus. Compared to other members of the sulfonylurea drug group, glipizide displays rapid absorption and onset of action with the shortest half-life and duration of action, reducing the risk for long-lasting hypoglycemia that is often observed with blood glucose-lowering agents. The chemical Formula of Glipizide is $C_{21}H_{27}N_5O_4S$.[3]

The present study was aimed to develop a simple, sensitive, rapid, precise, accurate, and validated bioanalytical method for the simultaneous estimation of Epalrestat and Pregabalin in human plasma. The developed method was validated according to US-FDAguidelines by using high-performance liquid chromatography.

MATERIALS AND METHODS

Materials

Blank human plasma, pure samples including Epalrestat, PregabalinandGlipizide were obtained from Spectrum Pharma Research Solutions, Hyderabad, India. HPLC grade acetonitrile was obtained from Merck Chemical Division, Mumbai. Analytical grade of orthophosphoric acid purchased from SD Fine Chemicals Ltd., Mumbai, India. The double distillation and purification with Milli-Q water purification system of purified water helped to prepare HPLC grade water.

Instrument

The analysis was performed by using Waters 2695 series HPLC comprised of vacuum degassing, auto-injector, and dual gradient pump with a photodiode array detector. The HPLC system was equipped with Empower 2 software.

Chromatographic Conditions

Drug samples were analyzed with Symmetry C18 150 X 4.6mm, 5µm column as stationary phase and was maintained at 30℃. The mobile phase was a mixture of acetonitrile and orthophosphoricacid buffer (0.1%) in the ratio of 55:45 v/v. The flow rate of the mobile phase was 1.0 mL/min and sample injection volume 10 µL. The detection of the effluents was carried out at 220 nm with a PDA detector. Samples of Epalrestat and Pregabalinwere prepared using water and acetonitrile diluent in 50:50 ratios.

Buffer preparation

1 mL of orthophosphoric acid was taken in a 1000 mL volumetric flask and make up the volume by using HPLC grade water to produce 1000mL.

Preparation of Epalrestat stock solution (0.5 mg/mL)

Take 50 mg of Epalrestat in a 100 mL volumetric flask and make the volume with diluent to produce 0.5 mg/mL.

Preparation of Pregabalin stock solution (0.3 mg/mL)

Take 30 mg of Pregabalin in 100 mL volumetric flask and make the volume with diluent to produce 0.3 mg/mL.

Preparation of Epalrestat spiking solutions (0.25 µg/mL to 10 µg/mL)

From the above Epalrestat stock solution 0.05mL, 0.01mL, 0.15mL, 0.8mL, 1.0mL, 1.2mL, 1.6mL and 2.0 mL was pipette and transferred to 8 individual 10 mL volumetric flask and make up the volume up to the mark with diluent to produce 2.5 µg/mL, 5.0 µg/mL, 7.5 µg/mL, 40 µg/mL, 50.0 µg/mL, 60 µg/mL, 80 µg/mL and 100 µg/mL.Calibration standards and quality control (QC) samples were prepared by spiking blank plasma with working stock dilutions of analytes to produce 0.25 µg/mL, 0.5 µg/mL, 0.75 µg/mL, 4.0 µg/mL, 5.0 µg/mL, 6.0 µg/mL, 8.0 µg/mL and 10.0 µg/mL.

Preparation of Pregabalin spiking solutions (0.015 µg/mL to 6.0 µg/mL)

From the above Pregabalin stock solution solution 0.05mL, 0.01mL, 0.15mL, 0.8mL, 1.0mL, 1.2mL, 1.6mL and 2.0 mL was pipette and transferred to 8 individual 10 mL volumetric flask and make up the volume up to the mark with diluent to produce 1.5 µg/mL, 3.0 µg/mL, 4.5 µg/mL, 24 µg/mL, 30 µg/mL, 36 µg/mL, 48.0 µg/mL and 60 µg/mL.Calibration standards and quality control (QC) samples were prepared by spiking blank plasma with working stock

dilutions of analytes to produce 0.15 µg/mL, 0.3 µg/mL, 0.45 µg/mL, 2.4 µg/mL, 3.0 µg/mL, 3.6 µg/mL, 4.8 µg/mL and 6.0 µg/mL.

Preparation of internal standard solution (1 µg/mL)

Take 10 mg of Glipizide in a 10 mL volumetric flask and make up the volume with diluent to produce 1000 µg/mL.From the above stock solutions, 0.5mL was pipetted out into a 10mL volumetric flask and then made up to the final volume with diluents to produce 500µg/mL. From the above solution, take 0.5mL of solution and spiking blank plasma with working stock dilutions of analytes to produce 10 µg/mL ISD concentration.

Method validation[4,5]

The analytical method was validated according to US-FDA guidelines for the following parameters

System suitability test

The system suitability test was performed before the analysis of every batch of samples to ensure the reproducibility of the chromatographic system.The HPLC system suitability test was performed by running six injections of diluted drugs and ISTD in the linear region of the calibration curve and measuring the percentage coefficient of variance (% CV).

Linearity

The linearity experiment was performed six times to check the detector response to the drug to be linear in function with various concentrations of Epalrestat and Pregabalin0.25-5.0 µg/mL and 0.16-3.25 µg/mL respectively. The working standards were prepared by adding different concentrations of Epalrestat and Pregabalinand a fixedconcentration of Glipizide (10 µg/mL) solution spiked in plasma to obtain the required concentration range. Samples were extracted and injected into the HPLC system. The drug /ISTD peak area ratio was plotted against the concentration of the drug and expressed in terms of coefficient of determination (r^2).

LLOQ (Sensitivity)

The lower limit of quantification (LLOQ) is the lowest concentration of analyte in a sample which can be quantified reliably, with acceptable accuracy and precision. The LLOQ is considered to be the lowest calibration standard. In addition, the analyte signal of the LLOQ sample should be at least 5 times the signal of a blank sample.

Precision and accuracy

Precision and accuracy of the developed method were determined by analysis of quality control (QC) samples at three different concentrations covering the low, medium and, higher ranges of the calibrations curve. Intra-day variation of the assay was done by injecting six samples for each concentration on the same day. Inter-day variation was assessed by injecting nine samples of each concentration (on 15 days) for two weeks. The precision of the method is expressed in terms of the percent coefficient of variance (% CV), and accuracy was expressed as a percentage of the theoretical concentration (Observed concentration / Theoretical concentration × 100).

Recovery

The recoveries for the Epalrestat, Pregabalinand,Glipizide(Internal standard) were determined by spiking known amounts of drugs into the drug-free human plasma to obtain three different concentrations covering the low, medium, and higher ranges of the calibration curves. Recoveries were determined by comparing the peak area of extracted QC samples with the peak area of recovery standards at the same nominal concentrations.

Specificity/Selectivity

The specificity was verified by checking the interference of endogenous compounds in human plasma at the retention time of the Epalrestat, Pregabalinand Glipizide(Internal standard) by evaluating six lots of plasma.

Ruggedness

The ruggedness was done by changing the person to person for linearity, precision, and accuracy in the levels of ULOQ, LQC, MQC, and HQC.

Stability

Stability studies were performed as zero hours, long term at -28ºC, and long term at -80ºC. Day zero having two samples with six replicates of high-quality control (HQC) and lower quality control (LQC) levels. Long term at -28ºC and long term at -80ºC have HQC and LQC level with % Stability finding by comparison sample and stability sample.

RESULTS AND DISCUSSION

Method development[6,7]

In the present study, acetonitrile was the solvent of choice, to obtain satisfactory values for recovery of Epalrestat and Pregabalin which showed good resolutions with no interferences

peak. Hence, extraction with acetonitrile was optimized as the sample treatment procedure. The mobile phase was optimized to provide sufficient selectivity towards the drugs. Orthophosphoric acids contribute high sensitivity and selectivity when compared with other buffers. The optimized mobile phase consisted of acetonitrile and 0.1% OPA buffer in the ratio of 55:45 v/v. The Injection volume was optimized to 10 µL. The column temperature was maintained at 30°C (ambient). Retention times were 3.828 ± 0.05 min for Epalrestat, 4.699 ± 0.05 min for Pregabalin and 2.463 ± 0.05 min for Glipizide (ISTD).The representative chromatogramof blank human plasma with the internal standardand Spiked plasma sample (HQC) analytes are shown in Fig. 4-5.

System suitability test

The number of area ratio, retention time, and peak areas was also determined as a means of validation parameter. The values obtained are listed in Table 1. The % CV calculated for the method was found to be less than 2%, which revealed the suitability of the developed method and the optimized chromatographic conditions. These values met the requirements of USP24/ NF19 46 and were therefore found to be satisfactory and the results of the study were shown in Table 1.

LLOQ (Sensitivity)

The sensitivity of the method was determined at 0.25 µg/mL of Epalrestat with a % CV of 2.73 and 0.160 µg/mL of Pregabalin with a % CV of 8.44. The data for LLOQ was presented in Table 2. The chromatogram of an extracted plasma sample spiked with 0.25 µg/mL and 0.160 µg/mL of Epalrestat and Pregabalin were revealed in Fig. 6.

Linearity

The linearity of the method was evaluated from the calibration curve of spiked plasma samples at several concentration levels of Epalrestat and Pregabalin (constructed for six consecutive days). The mean area ratio of the peak area of the drug to the peak area of the internal standard yielded a linear correlation over concentration ranges from 0.25-10.0 µg/mL and 0.160-6.5 µg/mL respectively. The method exhibited excellent linearity for this range. A typical calibration curve of spiked plasma samples with the regression equation and the respective correlation coefficient (r^2) of Epalrestat and Pregabalinwas shown in Fig. 7, Fig. 8 and, Table 3.

Precision and accuracy[8]

The precision of the method was measured by the percentage Coefficient of variance (% CV) over the concentration range of LQC, MQC and, HQC samples respectively of the drug during validation. Intra-day precision of the method ranged from 4.0 to 10.8% CV. Inter-day precision of the method was found to be 3.88 to 10.25% CV. Nominal values (%) for recovery of Epalrestat and Pregabalinfrom QC samples were tested intra-day and inter-days. Intra-day accuracy ranges from 99.64 to 100.88% whereas Inter-day accuracy ranges from 99.64 to 100.88%. Result from the determination of intra-day and inter-day, accuracy and precision are given in Table 4. Reproducibility of the developed assay method was observed on the same day and different days. The percentage coefficient of variance (% CV) was found to be less than 15% for both samples over the concentration range assayed.

Recovery

The recovery for the Epalrestat, Pregabalin and internal standard Glipizide were determined by spiking known amounts of drugs into a drug-free human plasma to obtain three different concentrations covering the low, medium and, higher ranges of the calibration curve. The samples were then extracted and analyzed as described earlier. The recovery was calculated by comparing the peak areas of the drugs with those obtained from pure standards in the mobile phase and ISTD in the mobilephase at the same concentration. The recovery of Epalrestat and Pregabalin ranges from 98.20±416.06 % to 98.28±0.21%, while the absolute recovery for ISTD was 97.92±417.39, the results of recovery studies were shown in Table 5.

Stability studies[9]

Stability studies were performed to evaluate the stability of Epalrestat and Pregabalinboth in aqueous solution and in plasma after exposure to various stress conditions. Epalrestat and Pregabalin were stable did not show any degradation when stored at different conditions. A low value of percentage difference (<15) between area ratio for stability test samples and fresh QC samples confirm the stability of drugs on Zero hours, long term at -28ºC and long term at -80ºC, results of LQC, MQC, and HQC were more than 85% which are within acceptable limits. The results of the stability study were given in Table 6.

CONCLUSION

The developed RP-HPLC bioanalytical method is an accurate, specific and, simple method for simultaneous determination of Epalrestat and Pregabalin. The method involves a simple extraction procedure, separation on a reversed-phase column with an internal standard and PDA detector. The validation data demonstrated good precision and accuracy, which proves the reliability of the proposed method. Thus the method suits routine therapeutic drug monitoring (TDM), specializes in the measurement of medication concentrations in blood for Epalrestat and Pregabalin. It is also helpful in pharmacogenetic, demographic, and clinical information, and/or on the posterior measurement of blood concentrations of drugs (pharmacokinetic monitoring) of Epalrestat and Pregabalin in human plasma. The present developed method could be adapted for the determination of bioavailability and bioequivalence required for filing NDA and ANDA.

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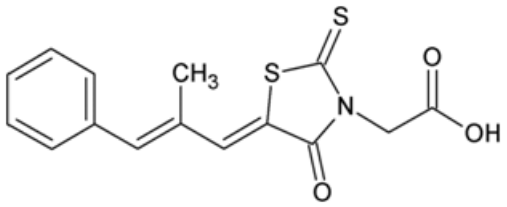

Fig. 1: Chemical Structure of Epalrestat

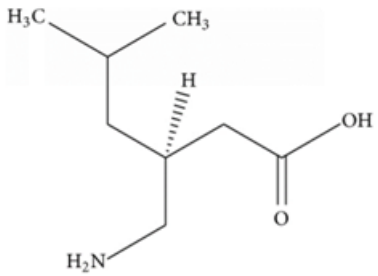

Fig. 2: Chemical Structure of Pregabalin

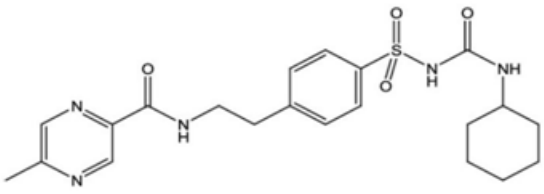

Fig. 3: Chemical Structure of Glipizide

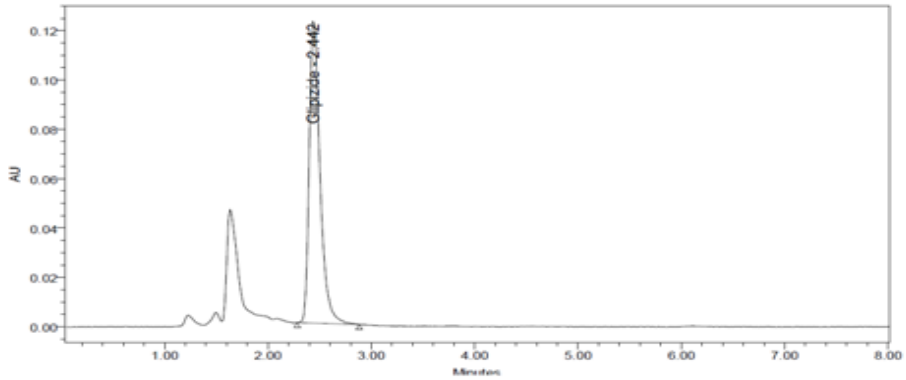

Fig. 4: Representative Chromatogram Human Plasma of Glipizide (ISTD)

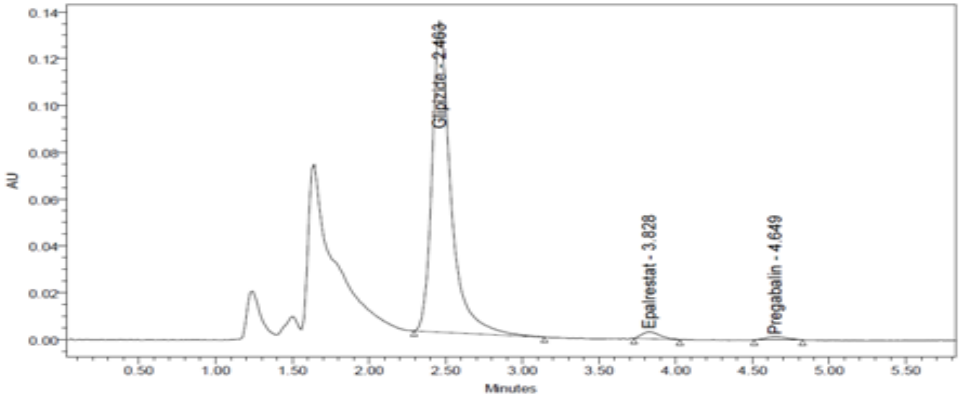

Fig. 5: A Representative Chromatogram of Spiked Plasma Sample (HQC) of analytes

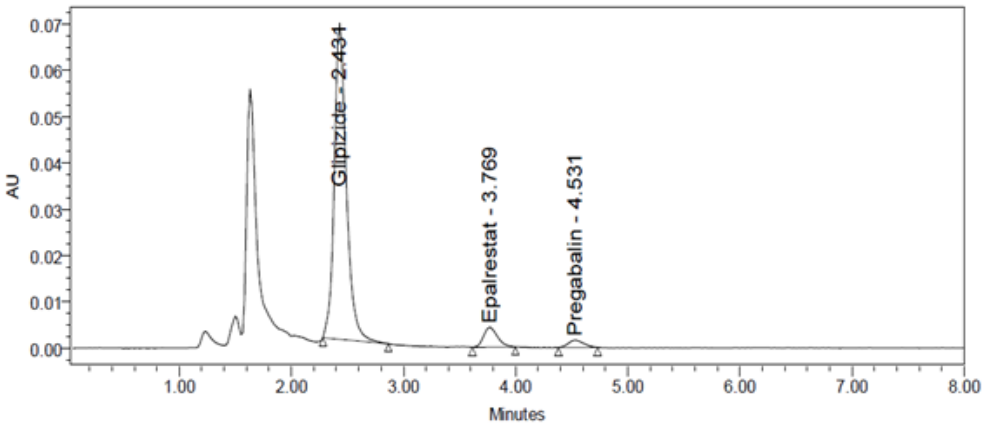

Fig. 6: A Representative Chromatogram of Spiked Plasma Sample (LLOQ) of analytes

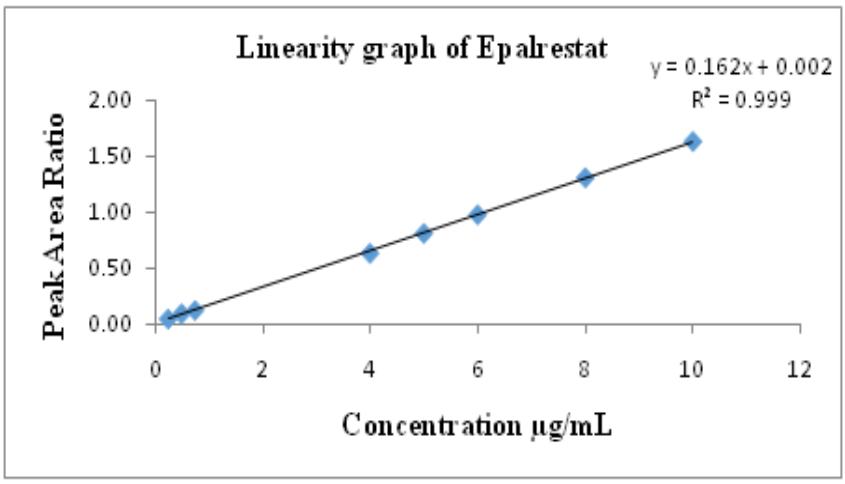

Fig. 7: Calibration Curve of Epalrestat

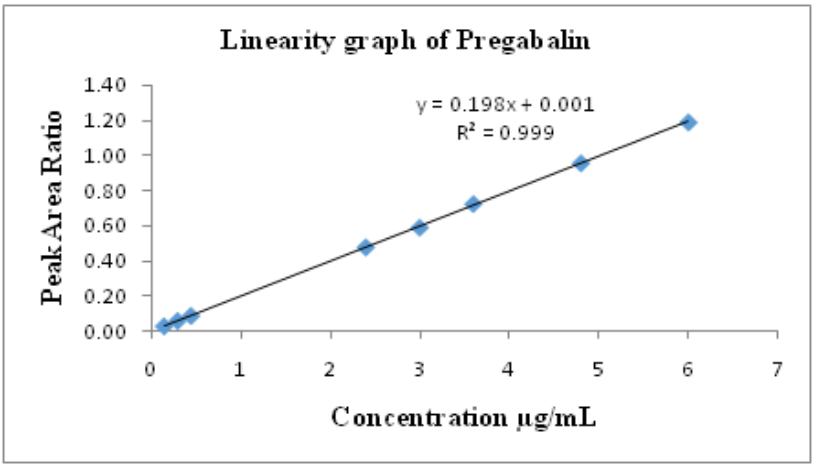

Fig. 8: Calibration Curve of Pregabalin

Table 1: System Suitability Data of Epalrestat and Pregabalin

S.No.	Epalrestat		Internal Standard		Peak Area ratio	Pregabalin		Internal Standard		Peak Area ratio
Sample Name	RT (Min)	Peak Area	RT (Min)	Peak Area		RT (Min)	Peak Area	RT (Min)	Peak Area	
AQMQC	3.765	41549	2.427	50563	0.8217	4.537	31049	2.427	50563	0.6141
	3.768	41696	2.429	50704	08223	4.544	29910	2.429	50704	0.5899
	3.770	40980	2.431	51536	0.7952	4.548	30610	2.431	51536	0.5940
	3.780	40967	2.434	50501	0.8112	4.549	29962	2.434	50501	0.5933
	3.785	41292	2.436	50124	0.8238	4.550	29830	2.436	50124	0.5951
	3.784	41069	2.439	50686	0.8103	4.555	30101	2.439	50686	0.5939
MEAN	3.775		2.433		081409	4.547		2.433		0.59670
SD	0.0081		0.0045		0.010968	0.0061		0.0045		0.008689
%CV	0.21		0.19		1.35	0.13		0.19		1.46

Acceptance Criteria: The % CV of the retention time (RT) should be ≤2.00 %.
The % CV of the area ratio should be ≤ 5.00%.

Table 2: Sensitivity Results of Lower Limit of Quantitation (LLOQ)

Sample /Parameter	Epalrestat		Pregabalin	
	Nominal Concentration 0.250 µg/mL		Nominal Concentration 0.160 µg/mL	
	Cal. Conc. (µg/mL)	% Accuracy	Cal. Conc. (µg/mL)	% Accuracy
LLOQ-1	0.252	100.8	0.162	101.25
LLOQ-2	0.246	98.4	0.158	98.75
LLOQ-3	0.249	99.6	0.148	92.5
LLOQ-4	0.245	98.0	0.151	94.3
LLOQ-5	0.254	101.6	0.171	106.87
LLOQ-6	0.235	94	0.185	115.62
Mean Cal. Conc.	0.2468		0.1625	
SD	0.00674		0.01372	
%CV	**2.73**		**8.44**	
% Mean Accuracy	**98.73**		**101.55**	

Acceptance Criteria: At least 67% (4 out of 6) of samples should be within 80.00-120.00%. %Mean accuracy should be within 80.00-120.00%. % CV accuracy should be ≤ 20.00%.

Table 3: Calibration Curve (Linearity) Data of Epalrestat and Pregabalin

Nominal Concentrations (µg/mL)	Epalrestat			Nominal Concentrations (µg/mL)	Pregabalin		
	*Mean	% CV	% Mean Accuracy		*Mean	% CV	% Mean Accuracy
0.25	0.240	9.19	98.40	0.160	0.1620	7.71	101.25
0.500	0.5013	1.92	100.27	0.320	0.3160	17.11	98.75
0.750	0.7447	1.14	99.29	0.480	0.4783	7.96	99.65
4.000	4.0617	1.26	101.54	2.600	2.6175	14.40	100.67
5.000	5.0333	1.35	100.67	3.250	3.2313	13.72	99.43
6.000	5.9037	2.14	98.39	3.900	3.9300	3.02	100.77
8.000	7.9963	2.15	99.95	5.200	5.2600	5.48	101.15
10.000	10.0737	1.48	100.74	6.500	6.5617	2.28	100.95
*Mean values represent three different samples for each concentration							

Acceptance Criteria: The regression coefficient should be R^2 = 0.0999.

Table 4: Precision and Accuracy Data of Epalrestat and Pregabalin

Drugs	Concentration added (µg/mL)	Recovery (%Mean±S.D)	Intra-Day (% CV)	Accuracy (%)	Recovery (%Mean±S.D)	Inter-Day (% CV)	Accuracy (%)
Epalrestat	0.250	0.2491±0.0181	7.59	99.64	0.2491**±0.0186**	7.49	99.64
	0.750	0.7565±0.0526	6.95	100.8	0.7565**±0.0508**	6.72	100.87
	5.000	3.535±0.2024	4.00	101.2	5.0628**±0.1966**	3.88	101.26
	8.000	7.915±0.6389	8.08	98.94	7.9153**±0.6128**	7.74	98.94
Pregabalin	0.160	0.1596±0.0172	10.8	99.76	0.1596**±0.0163**	10.25	99.76
	0.480	0.4842±0.0479	9.9	100.88	0.4842**±0.0451**	9.33	100.88
	3.250	3.2652±0.3357	10.3	100.46	3.2652**±0.3189**	9.77	100.47
	5.200	5.1881±0.3334	6.4	99.77	5.1881**±0.3236**	6.24	99.77

Mean values represent six different plasma samples for each concentration.
$Interday was determined from nine different runs over two weeks.
The concentration of each run was determined from a single calibration curve run on the first day of the study.
Acceptance Criteria: The within and between batch precision for LQC, MQC and, HQC Samples should be ≤15.00% and for the LLOQ QC, It should be ≤ 20.00%.

Table 5: Recovery Study Data of Epalrestat, Pregabalin and Internal standard Drugs from Human Plasma

Drugs	Concentration added (µg/mL)	Recovery (% Mean ± S.D)	% CV	Overall % CV
Epalrestat	0.750	98.42±58.18	0.93	0.49
	5.000	97.49±647.96	1.58	
	8.000	98.20±416.06	0.64	
Pregabalin	0.480	98±0.83	0.83	0.24
	3.250	97.81±0.41	0.41	
	5.200	98.28±0.21	0.21	
Internal standard	10.00	97.92±417.39	0.81	--

Acceptance Criteria:
The % CV of recovery at each QC level and for ISTD should be ≤ 15.00 %.
The overall mean recovery % CV for all QC levels should be ≤ 20.00 %.

Table 6: Stability Study Data of Epalrestat and Pregabalin

Sample	Epalrestat				Pregabalin			
	Nominal Concentration (µg/mL)	Mean calculated Conc. ± S.D (µg/mL)(n=6)	%CV	% Mean Accuracy	Nominal Concentration (µg/mL)	Mean calculated Conc. ± S.D (µg/mL) (n=6)	% CV	% Mean Accuracy
Stability at day Zero								
HQC	0.750	7.960**±0.510**	6.41	99.50	5.200	5.1232**±0.525**	10.26	98.52
LQC	8.000	0.7352**±0.066**	8.98	98.02	0.480	0.4732**±0.056**	11.950	98.58
Long term at -28ºC								
HQC	0.750	7.962**±0.220**	2.77	99.52	5.200	5.2155**±0.3546**	6.80	100.30
LQC	8.000	0.7503**±0.072**	9.56	100.04	0.480	0.4893**±0.0431**	8.82	101.4
Long term at -80ºC								
HQC	0.750	7.840**±0.665**	8.49	98.00	5.200	5.2343**±0.3670**	7.01	100.66
LQC	8.000	0.7493**±0.568**	7.57	99.91	0.480	0.4872**±0.0417**	8.57	101.49

Acceptance Criteria: At least 67% (8 out of 12) of total QC samples and 50% (3 out of 6) at each level should be within 85.00 - 115.00%. The %Mean accuracy of LQC and HQC should be within 85.00-115.00%. The % CV of LQC and HQC samples should be ≤ 15.00%.

REFERENCES

1. https://go.drugbank.com/drugs/DB15293.
2. https://go.drugbank.com/drugs/DB00230.
3. https://go.drugbank.com/drugs/DB01067.
4. Bioanalytical method validation-Guidance for industry, U.S. Department of health and human services. USFDA. 2018;1-40.
5. Sowjanya P. RP HPLC Validation of Pregabalin in Bulk and Dosage Form. Research & Reviews: Journal of Hospital and Clinical Pharmacy. 2016; 2(4): 75-90.
6. Kasawar GB and Farooqui MN. Development and Validation of HPLC Method for the Determination of Pregabalin in Capsules. Indian Journal of Pharmaceutical Sciences. 2010; 72(4): 517-519.
7. Krima RP, Dimal AS, Falgun AM, Usmangani KC and Kashyap KB. Liquid Chromatographic Estimation of Epalrestat and Methylcobalamin in Pharmaceutical Formulation. Research and Reviews: Journal of Pharmaceutical Analysis. 2014; 3(2): 35-42.
8. Mili P, Bhakti JL, Vijay M, Bhavesh SN, Hetal KP and Bhumi RP. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Epalrestat and Methylcobalamin in Tablet Dosage Form. World Journal of Pharmacy and Pharmaceutical Sciences. 2015; 4(5): 574-584.
9. Anil Mohan J, Rajkumar B Bhavya T and Ashok Kumar A. RP-HPLC Method Development and validation for the Simultaneous Quantitative Estimation of Pregabalin, Mecobalamin and Alpha Lipoic Acid in Capsules. International Journal of Pharmacy and Pharmaceutical Sciences. 2014; 6(1): 270-277.