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Development and Validation of Analytical Methods for Simultaneous Estimation of Pregabalin and Amitriptyline Hydrochloride in their Combined Marketed Dosage form

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ABSTRACT:

A reversed-phase liquid chromatographic method has been developed and validated for estimation of Pregabalin (PRE) and Amitriptyline hydrochloride (AMI) in Tablet dosage form. Chromatography was carried on C18 (25cm x 0.46 cm) Hypersil BDS analytical column using mobile phase Buffer (Potassium Dihydrogen Phosphate): Acetonitrile (55:45v/v) pH 4.0 with O-Phosphoric acid at a flow rate of 1.0 ml/min. The detection was carried out at 210 nm. The retention time of PRE and AMI was found to be 5.100 min and 3.227 min respectively. Assay result of marketed formulation of PRE and AMI was found to be 100.84% and 100.46% respectively. The proposed method was validated with respect to linearity, accuracy, precision, selectivity and robustness. Recovery of PRE and AMI was found to be 100.29% and 100.33% respectively. PRE and AMI were scanned in wavelength range of 200-400 nm. The proposed method for estimation of PRE and AMI were found to be simple, precise and accurate is applicable for simultaneous determination of PRE and AMI in marketed tablet formulation.

KEY WORDS: Pregabalin and Amitriptyline hydrochloride, RP-HPLC, Validation.

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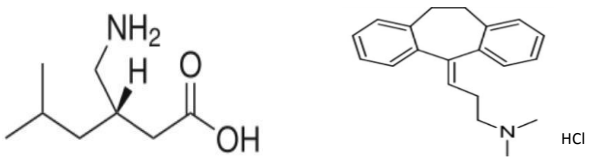
INTRODUCTION:

The IUPAC name of Pregabalin and Amitriptyline hydrochloride is (S)-3-(aminomethyl)-5-methylhexanoic acid and 3-(10, 11-Dihydro-5H-dibenzo[a,d]cycloheptene-5-ylidene)-N,N-dimethylpropan-1-amine respectively, with molecular formula C8H17NO2 and C20H24ClN respectively and molecular weight 159.23 and 313.9 g/mol respectively. The molecular structure of drug is given in Fig.1



Pregabalin Amitriptyline hydrochloride

Figure 1: Chemical structure

In this combination Pregabalin used as a anticonvulsant and analgesic used for neuropathic pain to treat the convulsion and Amitriptyline hydrochloride used as a antidepressant to treat the depression.

Pregabalin Official in IP-2014 and EP(8.7). Amitriptyline hydrochloride Official in IP-2014, BP-2016, USP 38 and EP(8.0). However no analytical method has been reported till date for the estimation of Pregabalin and Amitriptyline hydrochloride in their combined marketed dosage form using RP-HPLC method. The present paper describes the development and validation of analytical methods for simultaneous estimation of Pregabalin and Amitriptyline hydrochloride in their combined marketed dosage form. The proposed method are optimized and validated as per ICH guidelines.

MATERIAL AND METHODS:

Materials

HPLC Thermo Separation Product (TSP) UV 2000. UV-Visible Spectrophotometer (SIMADZU 1800) with software UV probe 2.0 (Simadzu Corporation, Kyoto, Japan). Pregabalin and Amitriptyline hydrochloride Purchased from Torrent Pharmaceutical Ltd and Sun Pharmaceutical Industries Ltd. The commercial fixed dose MAXGALIP-AT was procured from local market. All solvents were HPLC grade.

METHOD DEVELOPMENT

The working standard solutions of Pregabalin (75 ppm) and Amitriptyline hydrochloride (10 ppm) were prepared in Methanol. These drug solutions were than scanned in UV region of 200-400 nm and overlay spectrums were recorded. Maximum absorbance was obtained at 193.43nm and 240.48nm for PRE and AMI respectively. **210 nm** was taken as a detection wavelength for HPLC analysis. UV spectra of both drugs shown in Fig. 2.

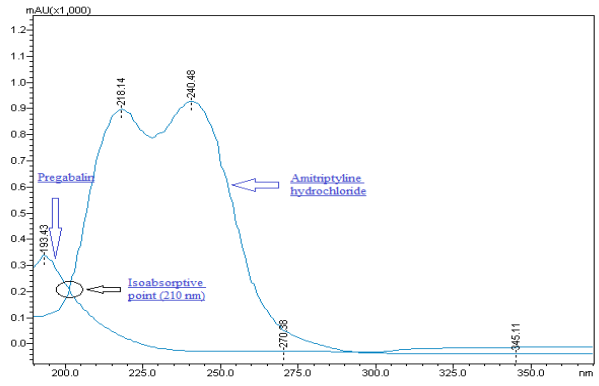


Figure 2: UV Spectra of Amitriptyline hydrochloride (10 ppm) and Pregabalin (75 ppm) prepared in Methanol

Selection of Mobile Phase

On the basis of various trails the mixture of Buffer (Potassium Dihydrogen Phosphate) at pH 4.0: Acetonitrile (55:45), at 1.0 ml/min flow rate, proved to be better than the other mixture in terms of peak shape, theoretical plate and asymmetry.

RP-HPLC

Table 1 System Suitability Parameters:

Parameters	Pregabalin	Amitriptyline hydrochloride
Retention Time	5.100	3.227
Theoretical Plates	7153	7121
Asymmetry	1.438	1.381
Resolution	9.585	

Preparation of Buffer

Take 6.8 gm Potassium dihydrogen phosphate into a 1000ml beaker. Add 800ml water and dissolve. Adjust pH 4.0 of this solution with 1% orthophosphoric acid. Make up volume with water upto 1000ml.

Preparation of mobile phase

Freshly Prepared buffer solution and Acetonitrile were taken and sonicate for 25 minute for degassing. Then fill both solutions in reservoir and set the volume to 55:45 v/v.

Preparation of test sample solution

Weigh 20 tablets accurately and powdered. A tablet powder equivalent to 75 mg of Pregabalin and 10 mg of Amitriptyline hydrochloride was taken into 100 ml volumetric flask. Add sufficient volume of mobile phase to dissolve and sonicate for 25 min. make up the volume up to mark with mobile phase and filter through whatman filter paper. From this above solution, suitable aliquots were transferred into 10 ml volumetric flask and volume was made up to the mark with mobile phase to get final concentration of 75 µland 10µl for Pregabalin and Amitriptyline hydrochloride respectively.

EXPERIMENTAL WORK

Linearity

The linearity for Pregabalin and Amitriptyline hydrochloride were assessed by analysis of combined standard solution in range of 37.5-112.5µg/ml and 5-15µg/ml respectively.

Accuracy (% Recovery)

Accuracy was determined over the range of 80%, 100%, and 120% of the sample concentration. Calculated amount of Pregabalin(PRE) and Amitriptyline hydrochloride (AMI) API were added in Drug solution to attain 80%, 100% and 120% of Amitriptyline hydrochloride and Pregabalin.

Precision

a) Method precision (Repeatability)

The precision of the instrument was checked by repeated injection (n=6) of standard solutions of 75µg/ml for Pregabalin and 10 µg/ml for Amitriptyline hydrochloride under the same chromatographic condition and measurement of peak area, retention time. The RSD value should not be more than 2%.

b) Intra-day precision

Variation of results within same day is called intra-day precision. The intra-day precision was determined for standard solution containing (37.5, 75, 112.5 µg/ml) for Pregabalin and (5, 10, 15 µg/ml) for Amitriptyline hydrochloride for the three times on the same day.

c) Interday precision

Variation of results amongst days is called inter-day precision. The inter-day precision was determined for standard solution containing (37.5, 75, 112.5 µg/ml) for Pregabalin and (5, 10, 15 µg/ml) for Amitriptyline hydrochloride for three times at different three days.

Limit Of Detection (LOD) and Limit Of Quantification (LOQ)

LOD and LOQ were calculated from the data from the linearity studies. For each replicate determination, Slope and intercept of the linearity plot were determined. Average of Slope (S) and standard deviation of the y intercept (σ) were calculated. From these values, the parameter LOD and LOQ were determined using following equation On the basis of response and slope of regression equation.

- LOD= 3.3 σ/s
- LOQ = 10 σ/s

Where, σ = the standard deviation of the response.

S = the slope of the calibration curve.

The slope S may be estimated from the calibration curve of the analyte.

Robustness

The robustness of proposed method was checked by changing the flow rate, mobile phase composition and pH ratio. The standard solution was injected three times for each varied conditions of flow and mobile phase ratio and chromatograms were recorded.

System suitability

System suitability parameter is established to ensure that the validity of the analytical method is maintained whenever used. Typical variations are the stability of analytical solution, different equipment and different analyzer. In case of liquid chromatography typical variations are pH of the mobile phase, the mobile phase composition, different lots or supplier of columns, the temperature and flow rate

Application of proposed method to the pharmaceutical dosage form

The method was applied to the pharmaceutical dosage form for determination of PRE and AMI. The proposed validated method was successfully applied for the routine simultaneous estimation of PRE and AMI in bulk powder.

RESULT AND DISCUSSION

Linearity

Calibration curve was found to be linear in the range of 37.5 - 122.5µg/ml for PRE and 5 - 15 µg/ml for AMI

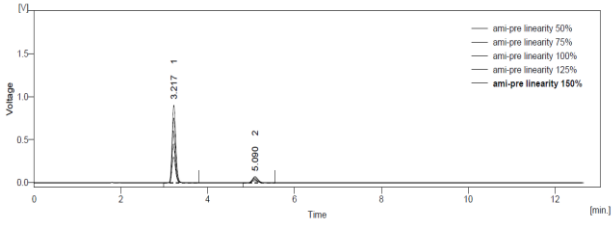


Figure 3 Linearity curve

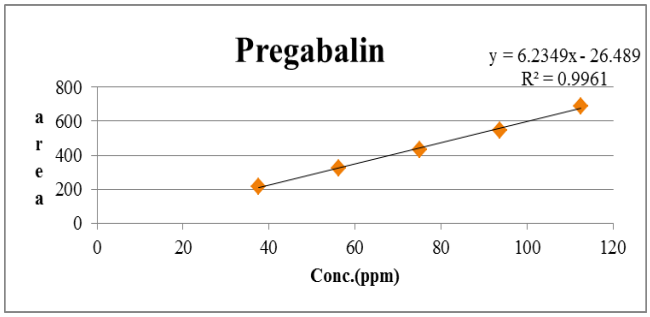


Figure 4 Calibration curve pregabalin

Table 2 Calibration curve data

Sr.No	Concentration(µg/ml)	Area	SD	%RSD
1	37.5	214.429	0.869	0.41
2	56.25	324.326	0.934	0.29
3	75	434.122	1.327	0.31
4	93.75	543.275	0.845	0.16
5	122.5	698.472	1.357	0.2

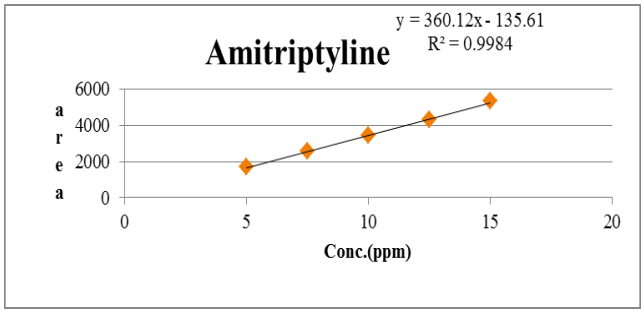


Figure 5 Calibration curve Amitriptyline

Table 3 Calibration curve data

Sr.No.	Concentration (µg/ml)	Area	SD	%RSD
1	5	1702.822	1.407	0.08
2	7.5	2562.733	1.11	0.04
3	10	3430.148	0.794	0.02
4	12.5	4293.186	0.618	0.01
5	15	5339.099	0.925	0.02

Accuracy

Accuracy was determined in terms of recovery study and the recoveries are done at three levels i.e 80%, 100%, and 120%. The data shows that the proposed method is accurate. % Recovery data obtained by the proposed method are shown in following table

Table 4 Data recovery study for Pregabalin

Accu-racy Level %	Amt. of API added	Net Area of Std.	Amt. Re-covered	% Recovery	Mean % Recovery	SD	%RSD
80%	30	172.724	29.83	99.417	100.631	1.123	1.12
	30	176.573	30.49	101.632			
	30	175.201	30.25	100.843			
100%	37.5	216.531	37.39	99.705	100.307	0.606	0.60
	37.5	219.161	37.84	100.916			
	37.5	217.822	37.61	100.300			
120%	45	261.573	45.17	100.371	99.945	0.392	0.39
	45	260.254	44.94	99.865			
	45	259.562	44.82	99.600			

Table 5 Data recovery study for Amitriptyline hydrochloride

Accu-racy Level %	Amt. of API added	Net Area of Std.	Amt. Re-covered	% Recovery	Mean % Recovery	SD	%RSD
80%	4	1363.001	3.95	98.774	99.657	0.775	0.78

	4	1379.598	4.00	99.976			
	4	1382.968	4.01	100.221			
100%	5	1708.869	4.95	99.070	99.610	0.686	0.69
	5	1731.500	5.02	100.382			
	5	1714.156	4.97	99.377			
120%	6	2110.841	6.12	101.979	101.738	0.332	0.33
	6	2098.029	6.08	101.360			
	6	2108.719	6.11	101.876			

Application of proposed method to the pharmaceutical dosage form (Tablet)

No interference of the excipients with the peaks of intrest appeared; hence the proposed method is applicable for the routine estimation of Pregabalin and Amitriptyline hydrochloride in pharmaceutical dosage forms.

Table 6 % Assay for MAXGALIP- AT

Brand Name	Drug	Amount taken (µg/ml)	Amount found (µg/ml)	% Assay ±SD
MAXGALIP-AT	Amitriptyline hydrochloride	10	10.04	100.46 ± 0.13
	Pregabalin	75	75.62	100.84 ± 0.25

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