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UV Spectrophotometric Methods for the Simultaneous Estimation of Pregabalin and Amitriptyline Hydrochloride in Combined Tablet Dosage Form

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Sunil More*, Ashpak Tamboli, Snehal Patil

Department of Pharmaceutical Chemistry, Sahyadri College of Pharmacy, Methwade, Sangola, 413307, Solapur, Maharashtra, India.

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ABSTRACT

The simple, accurate and precise UV spectrophotometric method has been developed for the simultaneous estimation of Pregabalin and Amitriptyline Hydrochloride in bulk and Maxgalip AT tablet dosage form. Two wavelengths 210 nm and 239 nm were selected for the estimation of Pregabalin and Amitriptyline Hydrochloride by simultaneous equation respectively. UV spectrophotometric method was developed and validated as per ICH guidelines using methanol as a solvent. Pregabalin and Amitriptyline Hydrochloride individually follows the Beer-Lamberts law over concentration range 37.5-187.5µg/ml and 5-20µg/ml, regression of coefficient was found to be r^2=0.996 and r^2=0.997 respectively. The percentage recovery was found in the range of 98% to 102% at three different levels. The proposed method was successfully applied for the determination of Pregabalin and Amitriptyline Hydrochloride in tablets dosage form as per ICH guidelines the result of the analysis were validated statistically and were found to be satisfactory.

INTRODUCTION

Pregabalin is chemically (*S*)-4-amino-3-(2-methyl propyl) butyric acid (Fig.1)[1 2]. It is an antiepileptic and structurally related to the neurotransmitter aminobutyric acid (GABA) it was recently approved for adjunctive treatment of partial seizures in adults in United States and Europe and for the treatment for neuropathic pain from post therapeutic neuralgia and diabetic neuropathy[3 4].

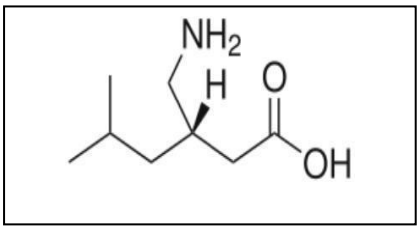

Fig.1- Structure of Pregabalin

Molecular Formula: $C_8H_{17}NO_2$

Molecular Weight: 159.229 g/mol

Amitriptyline hydrochloride is chemically 3-(10, 11-dihydro-5*H*-dibenzo [a, d] cycloheptene-5-ylidene)-*N*, *N*-dimethyl-1-propanamine hydrochloride (fig2)[2 5 6]. It is tricyclic antidepressant used in case of anxiety and also exerts an anticholinergic activity[7]. It works by increasing the levels of chemical messengers in the brain that help in regulating mood and treat depression. It also stops the movement of pain signals from nerves to the brain thereby relieving neuropathic pain[1 8].

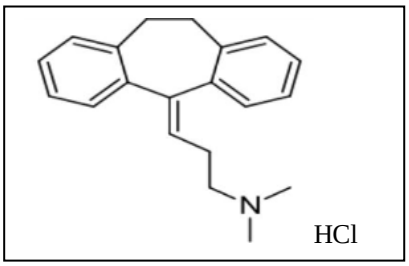

Fig. 2-Structure of Amitriptyline Hydrochloride

Molecular Formula: $C_{20}H_{24}ClN$

Molecular Weight: 313.869 g/mol

The objective of the present study was to develop new analytical UV spectrophotometry method and its validation parameters for the proposed method according to ICH guidelines for the estimation of Pregabalin and Amitriptyline Hydrochloride in tablets dosage form. Attempts were made to develop a simple, precise and accurate Simultaneous UV spectroscopic method[9 10 11 12].

MATERIALS AND METHODS

Chemical and reagents

Pregabalin and Amitriptyline Hydrochloride [bulk drug] used were of analytical reagent grade purchased from Unichem laboratories Ltd, Pharmaceutical Company in Goa Industrial Estate, Goa, India. Methanol (AR grade) was purchased from Research lab finechem. Industries Mumbai and double distilled water was used throughout the analysis.

Selection of solvent and wavelength

The absorbance of the drugs was found to be maximum in Methanol. So, Methanol is used as solvent and λmax of Pregabalin and Amitriptyline Hydrochloride was fixed as 210nm and 239nm respectively (Fig. 3).

Selection of Wavelength

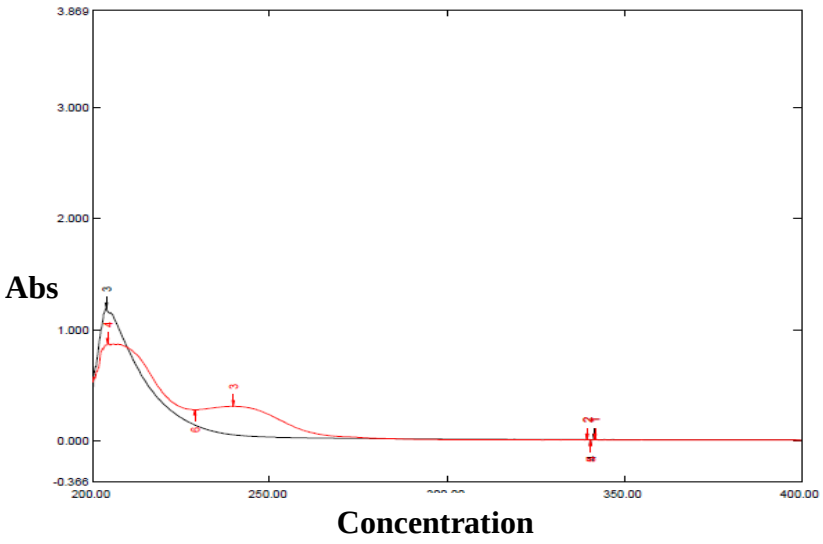

Fig. 3-Overlain spectra of Pregabalin and Amitriptyline Hydrochloride in Methanol

Instrumentation

Ashimadzu1800UV/VIS double beam spectrophotometer with 1cm matched quartz cells was used for all spectral measurements.

Preparation of standard stock solution

10mg of Pregabalin and 10mg of Amitriptyline hydrochloride were weighed accurately and transferred to a separate 10ml volumetric flask, dissolved in sufficient quantity of methanol then sonicated for 15min and diluted to 10ml with the same solvent so as to get the concentration of 1000µg/ml. From the respective standard stock solution, working standard solution was prepared containing 75µg/ml of PRGB and 10µg/ml of AMTR separately in mobile phase (Fig. 3).

Simultaneous equation method

In order to observe the feasibility of proposed method for simultaneous estimation of Pregabalin and Amitriptyline Hydrochloride in pharmaceutical formulations, the method was tried on standard mixture. Accurately weighed quantities of Pregabalin (10mg) and Amitriptyline Hydrochloride (10mg) were taken in 10 ml volumetric flask separately and dissolved in Methanol by vigorous shaking. The volume was made up to the mark using Methanol. The aliquot portions of above solution were further diluted with solvent to get Pregabalin (75ug/ml) and Amitriptyline Hydrochloride (10ug/ml). The absorbance of the resulting solutions was measured at 210nm and 239nm. Concentration of each drug was determined using simultaneous equation:

$$Cx = \frac{A2\ ay1 - A1\ ay2}{ax2\ ay1 - ax1\ ay2}$$

$$Cy = \frac{A1ax2 - A2ax1}{ax2\ ay1 - ax1\ ay2}$$

Where,

Cx = concentration of Pregabalin

Cy = concentration of Amitriptyline Hydrochloride

ax1 = absorptivity value of Pregabalin at 210 nm.

ax2 = absorptivity value of Amitriptyline Hydrochloride at 239 nm.

ay1= absorptivity value of Pregabalin at 210 nm.

ay2= absorptivity value of Pregabalin 210 nm.

A1 = absorbance of standard mixture at 210 nm.

A2 = absorbance of standard mixture at 239 nm.

Analysis of marketed formulation

Ten tablets of brand name Maxgalip AT were used. A quantity of tablet powder equivalent to Pregabalin (75mg) and Amitriptyline Hydrochloride (10mg) was transferred to 10ml volumetric flask and dissolved in methanol. The aliquot portion of filtrate was further diluted to get Pregabalin (75µg/ml) and Amitriptyline Hydrochloride (10µg/ml) respectively. The results obtained are shown in (Table 8).

Table 8: Analysis of marketed formulation

Sr. no.	PRGB			AMTR		
	Absorbance	**Amount recovered (µg/ml)**	**% recovery**	**Absorbance**	**Amount recovered (µg/ml)**	**% recovery**
1	0.342	73.750	98.33	0.380	9.737	97.368
2	0.338	72.750	97.000	0.391	10.026	100.263
3	0.351	76.000	101.333	0.382	9.789	97.895
4	0.347	75.000	100.000	0.390	10.000	100.000
5	0.349	75.500	100.667	0.389	9.974	99.737
6	0.340	73.250	97.667	0.396	10.158	101.579
Mean	0.345	74.375	99.167	0.388	9.947	99.474
%RSD	1.52	1.76	1.76	1.537	1.57	1.578

Validation of the Method

The proposed method was validated for various parameters such as linearity, precision, accuracy, Limit of detection (LOD), Limit of Quantitation (LOQ) according to ICH Q2 (R1) guidelines.

1. Linearity

Pregabalin was found to be linear in the range of 37.5-187.5µg/ml and Amitriptyline Hydrochloride was found to be linear in the range of 5-25µg/ml. The absorbance of this solution measured at 210 and 239 nm. Calibration curves were plotted using concentration Vs absorbance and the slop, intercept and correlation coefficient were calculated. The linearity values were shown in Table 1(Fig. 4 & 5).

Table 1: Linearity values of PRGB and AMTR

Parameter	PRGB	AMTR
Range	37.5-187.5µg/ml	5-25µg/ml
Slop	0.004	0.038
Intercept	0.047	0.010
Correlation coefficient	0.996	0.997

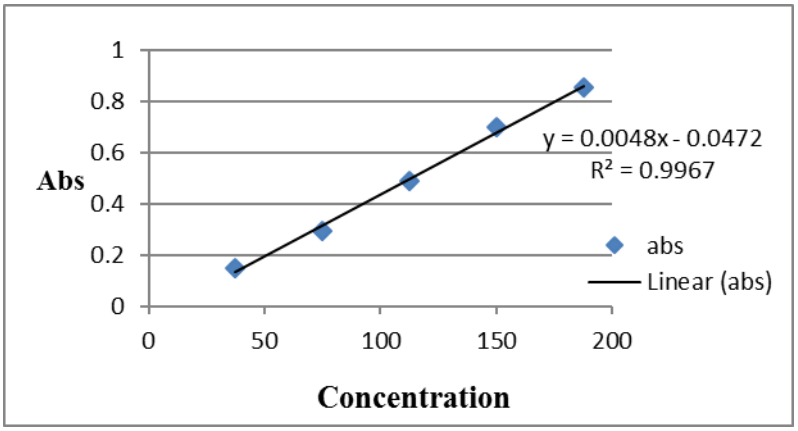

Fig. 4- Linearity graph of Pregabalin

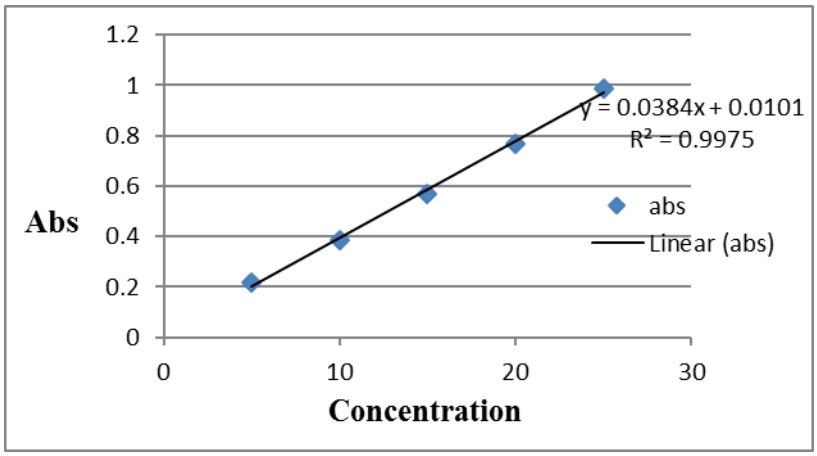

Fig. 5- Linearity graph of Amitriptyline Hydrochloride

2. Precision

Precision studies were carried in terms of intra-day and inter-day, the % relative standard deviation (%RSD) values were found to be less than 2, which indicate that the method is accurate. The results for intra-day precision are shown in (Table 2&3) and for inter-day precision are shown in (Table 4&5).

Table 2: Intra-day precision study of Pregabalin

Conc. µg/ml	Absorbance			Mean Absorbance	SD	%RSD
	Trial 1	Trial 2	Trial 3			
75	0.289	0.284	0.291	0.288	0.003606	1.251928
112.5	0.479	0.487	0.472	0.479	0.007506	1.565832
150	0.685	0.694	0.676	0.685	0.009	1.313869

Table 3: Intra-day precision study of Amitriptyline Hydrochloride

Conc. µg/ml	Absorbance			Mean Absorbance	SD	%RSD
	Trial 1	Trial 2	Trial 3			
10	0.383	0.376	0.386	0.381	0.005033	1.319901
15	0.568	0.562	0.572	0.567	0.005033	0.887172
20	0.768	0.775	0.762	0.768	0.006506	0.846821

Table 4: Inter-day precision study of Pregabalin

Conc. µg/ml	Absorbance			Mean Absorbance	SD	%RSD
	Trial 1	Trial 2	Trial 3			
75	0.295	0.298	0.301	0.298	0.003	1.006711
112.5	0.489	0.493	0.498	0.493	0.004509	0.914037
150	0.695	0.702	0.690	0.695	0.006028	0.866466

Table 5: Inter-day precision study of Amitriptyline Hydrochloride

Conc. µg/ml	Absorbance			Mean Absorbance	SD	%RSD
	Trial 1	Trial 2	Trial 3			
10	0.394	0.286	0.390	0.390	0.004	1.025641
15	0.578	0.582	0.588	0.582	0.005033	0.863825
20	0.784	0.790	0.792	0.788	0.004163	0.527895

3. Accuracy (Recovery studies)

To check the accuracy of the developed method and to study the interference of formulation additives, analytical recovery experiment was carried out by standard addition method**.** The recovery studies were carried out in three levels *i.e.* 50%, 100%, 150%, to assure the reliability of the above method recovery studies were carried out by mixing a known quantity of the standard drug with the preanalyzed sample formulation and the contents were reanalyzed by the proposed method. The recovery values were within the limits indicating that the method is accurate. The % recovery values were shown in the (Table 6&7).

Table 6: Recovery studies of PRGB

Level	Conc. (µg/ml)		Absorbance	% Recovery	Mean % Recovery ± RSD
	Sample	Std.			
50 %	75	37.5	0.495	99.556	98.444 ± 1.129
			0.490	98.444	
			0.485	97.333	
100 %	75	75	0.659	102.000	101.222 ± 0.829
			0.655	101.333	
			0.649	100.333	
150 %	75	112.5	0.811	101.867	101.867 ± 0.524
			0.815	102.400	
			0.807	101.333	

Table 7: Recovery studies of AMTR

Level	Conc. (µg/ml)		Absorbance	% Recovery	Mean % Recovery ± RSD
	Sample	Std.			
50 %	10	5	0.575	99.123	99.006 ± 0.892
			0.579	99.825	
			0.569	98.070	
100 %	10	10	0.778	101.053	100.877 ± 0.669
			0.771	100.132	
			0.781	101.447	
150 %	10	15	0.973	101.368	101.719 ± 0.363
			0.976	101.684	
			0.980	102.534	

4. Limit of detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S respectively. Where (σ) is the standard deviation of the response (y-intercept) and (S) is the mean of the slop of calibration plot. The

LOD values of PRGB and AMTR was found to be 15.81764µg/ml. and 1.515057µg/ml. respectively and the LOQ values were found to be 47.93224µg/ml. and 4.591081µg/ml.

RESULTS AND DISCUSSION

The present work provides an accurate, rapid, sensitive method for the simultaneous analysis of PRGB & AMTR in bulk and tablet formulation. Linear relationships between drug concentrations were obtained over the range of at 37.5-187.5&5-25µg/ml for PRGB and AMTR respectively. The correlation coefficient, slope and intercept obtained for each drug is as shown in Table 1. The proposed method was also successfully applied to a pharmaceutical formulation. The precision of the method with intra-day and inter-day precision was found to be good with % RSD less than 2, indicate that method was precise and the results presented in Table 2, 3, 4 and 5. Recovery studies results are tabulated in Table 6&7. For Pregabalin percent recovery ranged from 98.444% to 101.867%, with RSD ranging from 0.524 to1.129. For Amitriptyline Hydrochloride percent recovery ranged from 99.006% to 101.719 %, with RSD ranging from 0.363 to0.892.The % assay was found to be 99.167% for PRGB and 99.474% for AMTR. The assay results are shown in Table 8. The LOD and LOQ were found to be 15.81µg/ml and 1.51µg/ml, 47.93µg/ml and 4.59µg/ml for Pregabalin and Amitriptyline Hydrochloride, respectively. No interference was found in the spectrogram of the formulation within the absorbance indicating that excipients used in tablet formulation did not interfere with the simultaneous estimation of the Pregabalin and Amitriptyline Hydrochloride by the proposed UV spectroscopic method.

CONCLUSION

The recently developed UV spectrophotometric method for the determination of Pregabalin and Amitriptyline Hydrochloride simultaneously is simple, specific, accurate, precise, rapid and economical which indicates its competence for routine pharmaceutical analysis of Pregabalin and Amitriptyline Hydrochloride in bulk drug and marketed formulations. It is concluded that HPLC method is successfully utilized for the estimation of Pregabalin and Amitriptyline Hydrochloride. This new method has been successfully applied for routine analysis.

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