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ORIGINAL ARTICLE **OPEN ACCESS**

Development and Validation of HPTLC Method for The Estimation of Pregabalin and Duloxetine Hydrochloride in Bulk Drugs

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ABSTRACT

To method develop and validate simple, rapid, cost effective, linear, accurate, precise and economical for the estimation of Pregabalin and Duloxetine Hydrochloride in bulk and tablet dosage form by using HPTLC Method. Using mobile phase Methanol: Toluene: Ethyl Acetate: Formic Acid (3.0: 3.0: 6.0: 0.1). The method based on measurement of the standard solutions of these drugs were scanned over the spectrum and 246 nm. Linearity was observed in the range of 50-150% of the standard concentrations for both of the drugs. The regression equation and correlation coefficient (R2) of PG was found to be Y= 0.0018x + 0.0047 and 0.9988, and for DXH is Y=0.0025x+0.009, and 0.9979 respectively. The recovery analysis for PG was found to be 99.67, 98.70, 101.16, 100.56 and for DXH 98.77%, 98.30%, 99.81%, 99.95%. The % RSD was in the limited range of <2% suggesting that the said method is reproducible. Robustness parameters i.e. Change in saturation time of chamber by +5 min, -5 min and altering constitution of mobile phase by +10% and -10% showed that the method that is developed is stable when triggered by minute changes. Thus, the developed method and validation parameter were found to be within the limits as per ICH guidelines.

Keywords: *Pregabalin, Duloxetine Hydrochloride, HPTLC, linearity, accuracy, precision.*

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INTRODUCTION

The pharmacologically active S-enantiomer of a racemic 3-isobutyl gamma amino butyric acid analogue is Pregabalin, also known as (S)-3-(aminomethyl)-5-methylhexanoic acid. It is a well-known analgesic and anticonvulsant drug. pregabalin has been shown to be a highly effective adjunctive therapy for partial seizures in clinical trials.[1-2].

Depression and anxiety are both treated with Duloxetine Hydrochloride. It is moreover utilised to treat discomfort brought on by diabetes-related nerve degeneration (diabetic peripheral neuropathy). Duloxetine Hydrochloride is also used to treat fibromyalgia, which causes chronic (long-lasting) pain in the muscles and bones as well as muscle and joint stiffness and pain. [3-5].

Analytical method development and then method validation is a crucial process in the discovery of drug. Even if the drug is potential enough, it will face difficulties in entering the market if there are lack of validated methods. After the literature review, It was found that there is no work carried out on this combination of Pregabalin and Duloxetine Hydrochloride in analytical research studies. Hence, we selected this combination for studies [6,7,8].

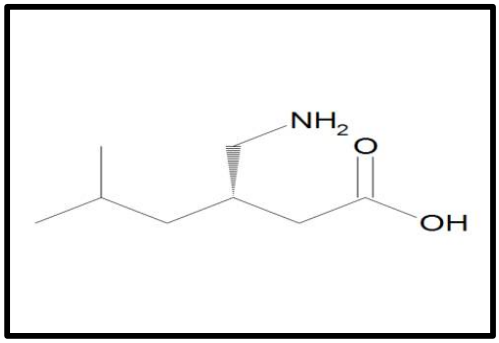

Fig. 1. Pregabalin

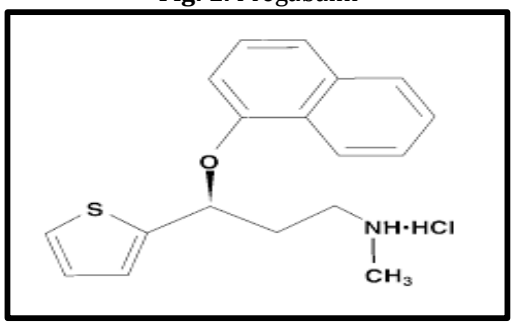

Fig. 2. Duloxetine Hydrochloride

MATERIAL AND METHODS

Chemicals

Pregabalin Gift sample by CTX Lifesciences Pvt Limited, Gujrat. And Duloxetine Hydrochloride gifted by Arti Pharma, Mumbai , Drug formulation Pregabid D 50/20 were obtained from the market, High Performance Thin Layer Chromatography (HPTLC) CAMAG – 151015,CAMAG Linomat 5 applicator , 100 µL syringe (Hamilton) Vision CAT 3.1.21109.3 software.

Chromatographic Condition

Stationary Phase Silica Gel 60 F254 (Merck), Mobile Phase- Methanol: Toluene: Ethyl Acetate: Formic Acid (3.0: 3.0: 6.0: 0.1), saturation time- 20 min Densitometric scanning performed at 246 nm.

EXPERIMENTAL WORK

HPTLC Method

Selection of mobile phase and chromatographic conditions:

Studies were carried out by using various combinations of solvents in various proportionson normal TLC plates to yield optimum parameters. It was noted that Methanol: Toluene : Ethyl Acetate : Formic Acid (3.0: 3.0: 6.0: 0.1)was most suitable because it gave good peak parameters. The optimised saturation time for the chamber was 20 min.TLC plate was dried using current of warm air by a dryer. Densitometric scanning was performed at 246 nm by Vision CAT 3.1.21109.3 software.[9]

Preparation of stock solution for standard:

Standard stock solution of PG was made by dissolution of 50 mg of drug in 10 ml of diluents (50:50/ Methanol: Water) to get concentration of 500 µg/ml.

Standard stock solution of DXH was made by dissolution of 20 mg of drug in 10 ml of diluents (50:50/Methanol: Water) to get concentration of 200 µg/ml. [10]

Preparation of standard solution for working:

Working solution of PG was formulated by mixing 1 ml of above standard stock solutionupto 10 ml solvent to give 50 µg/ml.

Working solution of DXH was formulated by mixing 1 ml of above standard stock solution upto 10 ml solvent to give 20 µg/ml. **[11]**

Preparation of sample solution:

Twenty tablets of the marketed preparation Pregabid D 50/20 which contain 50 mg of PG and 20 mg of DXH were accurately weighed and triturated well. Equivalent weight of 50 mg of Pregabalin and 20 mg Duloxetine Hydrochloride was taken and volume was made upto 10 ml with the solvent. Sonicated for 15 minutes. The solution was then filtered and diluted to obtain the sample solution in the concentration ratio of 5:2. [12]

Selection of wavelength:

246 nm was selected as the optimum wavelength by scanning the sample solution through400 nm– 200

nm. [13]

Method Validation[14-16]:

ICH Q2B guidelines for Linearity, Accuracy, Precision and Robustness were referred for validation.

RESULTS AND DISCUSSION

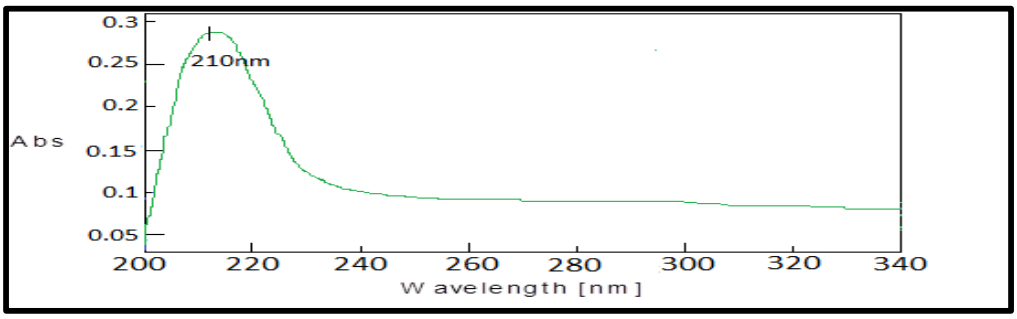

Fig. 3. λ max of Pregabalin

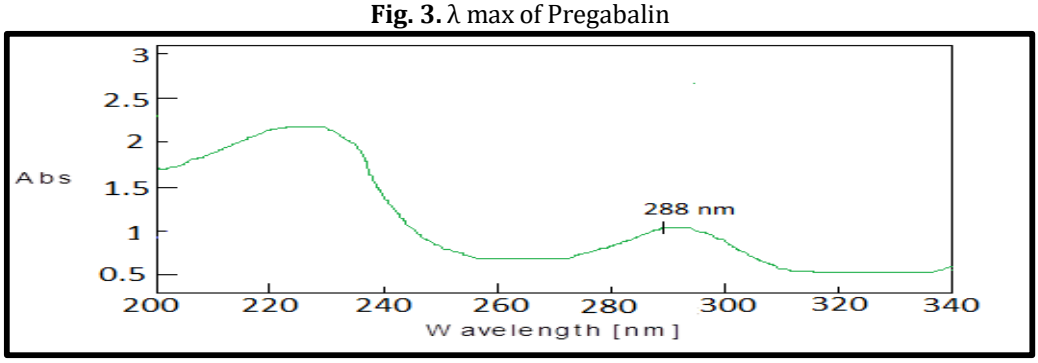

Fig. 4. λ max of Duloxetine Hydrochloridd

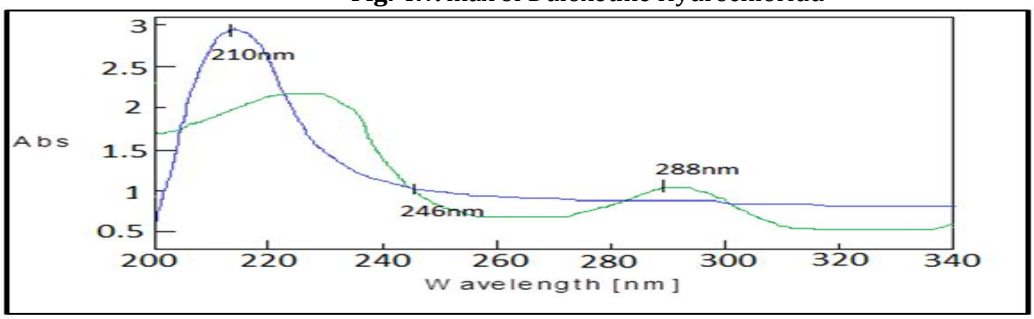

Fig. 5. Iso-absorptive point of Pregabalin and Duloxetine Hydrochloride

HPTLC method

a. After scanning the solutions of both drugs across the spectrum, it was discovered that the wavelength at which both drugs displayed a significant amount of absorbance was at 246 nm. As a result, this wavelength was chosen for analysis.

b. Densitogram for PG (1) and DXH (2)

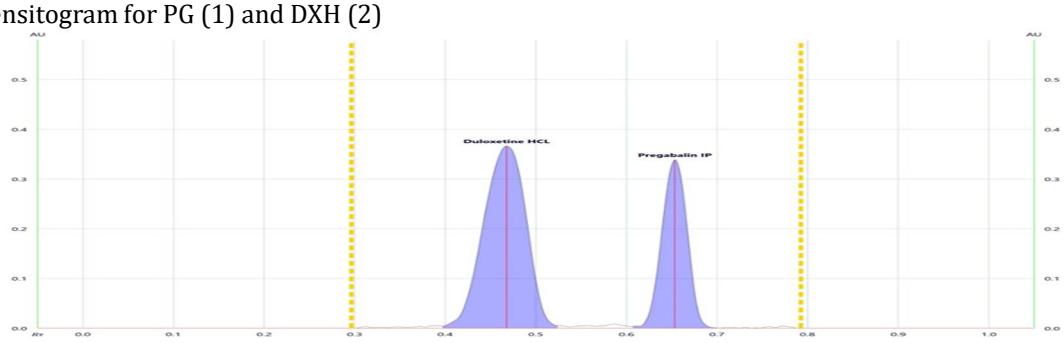

Fig. 6. Densitogram of PG and DXH

Method Validation

1. Linearity:

Table 1. Linearity for PG

Level	Standard Area of PG	Mean Standard Area of PG
50% level	0.00645	0.00641± 0.0005
	0.00635	
	0.00643	
75% level	0.00845	0.00840±0.0008
	0.00829	
	0.00843	
100% level	0.01009	0.01015±0.0006
	0.01022	
	0.01017	
125% level	0.01170	0.01177±0.0006
	0.01181	
	0.01182	
150% level	0.01345	0.01332±0.002
	0.01390	
	0.01335	

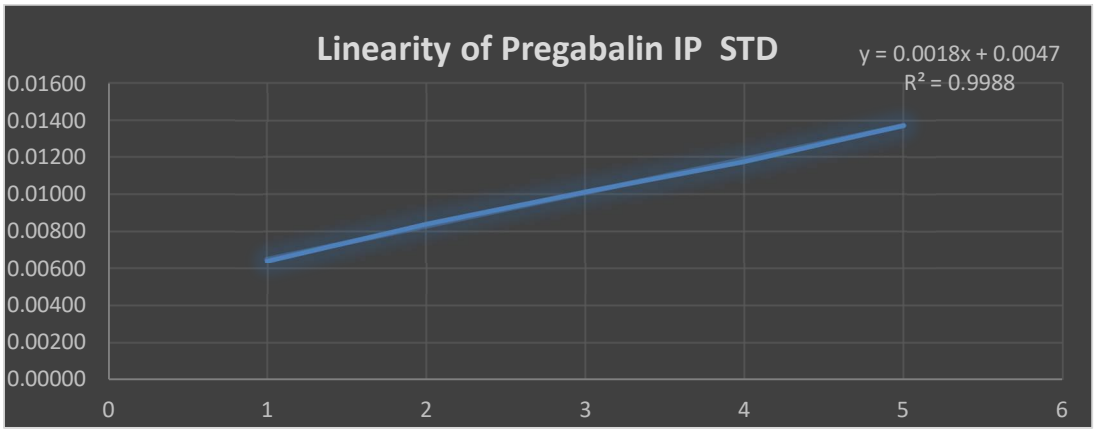

Fig. 7. Calibration curve of Pregabalin at 246 nm

Table 2. Linearity for DXH

Level	Standard Area of DXH	Mean Standard Area of DXH
50% level	0.01132	0.01135±0.0007
	0.01126	
	0.01141	
75% level	0.01425	0.01412±0.002
	0.01376	
	0.01422	
100% level	0.01671	0.01678±0.001
	0.01693	
	0.01683	
125% level	0.01869	0.01887±0.001
	0.01892	
	0.01897	
150% level	0.02111	0.02151±0.004
	0.02194	
	0.02156	

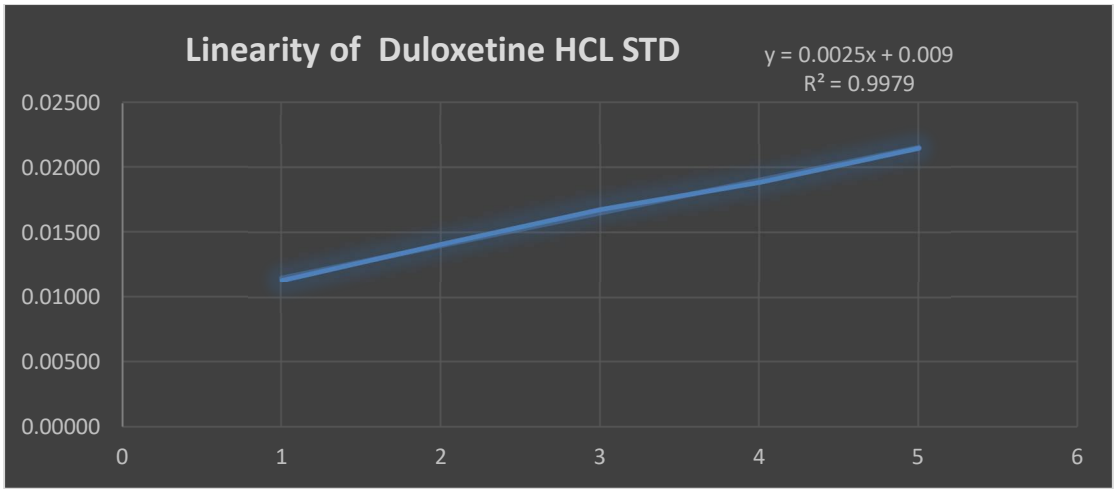

Fig. 8. Calibration curve of Duloxetine HCL at 246 nm

Accuracy

Table 3. Accuracy for PG

Sr. No	Spike level	Amt of PG added (mg)	Area of PG	Mean Area of PG	Amt. of PG Found (mg)	% Recovery of PG	Mean % recovery of PG	RSD %
1	100% Base	0.5	0.00903	0.0091	0.00	9.12	9.67	0.93
2	100%Base		0.00919			00.33		
3	100%Base		0.00914			9.57		
4	10 % Spike	0.5	0.00985	0.0099	0.493	8.10	8.70	0.64
5	10 % Spike		0.00999			9.15		
6	10 % Spike		0.00995			9.85		
7	20 % Spike	1	0.01117	0.0111	0.012	00.98	01.16	0.25
8	20 % Spike		0.01114			01.44		
9	20 % Spike		0.01103			01.07		
10	30 % Spike	0.5	0.01195	0.0120	0.508	00.51	00.56	0.06
11	30 % Spike		0.01198			00.60		
12	30 % Spike		0.01202			00.55		

Table 4. Accuracy for DXH

Sr. No	Spike level	Amt of DXH added (mg)	Area of DXH	Mean Area of DXH	Amt. of DXH Found	% Recovery of DXH	Mean % recovery of PG	RSD %
1	100% Base	0	0.01463	0.0147	0.00	98.31	98.77	0.92
2	100%Base		0.01477			99.41		
3	100%Base		0.01475			99.92		
4	10 % Spike	0.18	0.01588	0.0159	0.177	98.54	98.30	0.18
5	10 % Spike		0.01590			98.18		
6	10 % Spike		0.01596			98.18		
7	20 % Spike	0.4	0.01777	0.0178	0.399	99.79	99.81	0.20
8	20 % Spike		0.01782			99.95		
9	20 % Spike		0.01784			99.69		
10	30 % Spike	0.6	0.01943	0.0193	0.600	100.96	99.95	1.43
11	30 % Spike		0.01911			98.91		
12	30 % Spike		0.01937			99.98		

Precision

Table 5. Precision for PG and DXH

Sr. No.	Sample Area of Duloxetine HCL	Sample Area of Pregabalin IP	Assay of Duloxetine HCL	Assay of Pregabalin IP
1	0.02144	0.01148	100.91	99.09
2	0.02115	0.01155	99.55	99.69
3	0.02144	0.01164	100.91	100.47
4	0.0214	0.0117	100.72	100.98
5	0.02155	0.01165	101.43	100.55
6	0.02147	0.01153	101.05	99.52
Mean	0.02141	0.01159	100.71	100.16
SD	0.00014	0.00008	0.70	0.76
% RSD	**0.64**	**0.66**	**0.69**	**0.69**

Robustness

Chamber saturation time - +5min

Table 6. Robustness Study 1

Sr. No.	Standard Area of Duloxetine HCL STD	Rf of Duloxetine HCL STD	Standard Area of Pregabalin	Standard Area of Pregabalin
1	0.01741	0.415	0.00749	0.632
2	0.01718	0.423	0.00750	0.631
3	0.01692	0.423	0.00742	0.623
4	0.01714	0.423	0.00734	0.627
5	0.01698	0.423	0.00742	0.621
Mean	0.01713	0.421	0.00743	0.627
SD	0.00019	0.004	0.00006	0.005
% RSD	1.12	0.85	0.87	0.77

Chamber saturation time -5min

Table 7. Robustness Study 2

Sr. No.	Standard Area of Duloxetine HCL STD	Rf of Duloxetine HCL STD	Standard Area of Pregabalin	Standard Area of Pregabalin
1	0.01738	0.408	0.00746	0.627
2	0.01716	0.416	0.00755	0.626
3	0.001718	0.429	0.00751	0.619
4	0.01729	0.413	0.00738	0.627
5	0.01711	0.421	0.00743	0.619
Mean	0.01721	0.417	0.00743	0.624
SD	0.00012	0.008	0.00007	0.004
% RSD	0.70	1.92	0.89	0.68

Mobile phase composition +10%

Table 8. Robustness Study 3

Sr. No.	Standard Area of Duloxetine HCL STD	Rf of Duloxetine HCL STD	Standard Area of Pregabalin	Standard Area of Pregabalin
1	0.01485	0.411	0.00804	0.629
2	0.01453	0.421	0.00797	0.627
3	0.01431	0.422	0.00803	0.621
4	0.01452	0.423	0.00786	0.619
5	0.01452	0.411	0.00790	0.619
Mean	0.01455	0.418	0.00796	0.623
SD	0.00019	0.006	0.00008	0.005
% RSD	1.33	1.45	0.99	0.75

Mobile phase composition -10%

Table 9. Robustness Study 4

Sr. No.	Standard Area of Duloxetine HCL STD	Rf of Duloxetine HCL STD	Standard Area of Pregabalin	Standard Area of Pregabalin
1	0.02097	0.456	0.01227	0.608
2	0.02092	0.466	0.01235	0.615
3	0.02095	0.471	0.01260	0.619
4	0.02076	0.479	0.01260	0.626
5	0.02049	0.471	0.01238	0.626
Mean	0.02082	0.469	0.01241	0.619
SD	0.00020	0.008	0.00017	0.008
% RSD	0.97	1.80	1.39	1.24

DISCUSSION

The said estimation method of PG and DXH was worked upon on CAMAG made HPTLC apparatus. After many trials and errors the solvent and mobile phase selected mobile phase were Methanol: Toluene: Ethyl Acetate: Formic Acid (3.0: 3.0: 6.0: 0.1). The above solvent combination showed the best densitogram spectra on HPTLC by resolving into sharp peaks for both the drugs as well as in combination. [17-20] The standard solutions of these drugs were scanned over the spectrum and 246 nm was selected as the wavelength for analysis because it showed considerable amount of absorbance for both the drugs. Linearity was observed in the range of 50-150% of the standard concentrations for both of the drugs. The regression equation and correlation coefficient (R2) of PG was found to be Y= 0.0018x + 0.0047 and 0.9988, and for DXH is Y=0.0025x+0.009, and 0.9979 respectively. The recovery analysis for PG was found to be 99.67, 98.70, 101.16, 100.56 and for DXH 98.77%, 98.30%, 99.81%, 99.95%. The % RSD was in the limited range of <2% suggesting that the said method is reproducible. The accuracy for single drug 98.72% this combination give results more accurate. [21-22] Precision is commonly performed at three different levels, namely repeatability, intermediate precision, and reproducibility. Robustness parameters i.e. Change in saturation time of chamber by +5 min, -5 min and altering constitution of mobile phase by +10% and -10% showed that the method that is developed is stable when triggered by minute changes. % RSD studies also revealed that the method that is developed is reproducible for all the analytical wings.

CONCLUSION

The HPTLC method was examined to be rapid, precise, accurate and reproducible. No interference was noted in routine analysis of the drug on the Said method. The process was well employed for determining the drugs in their pharmaceutical dosage forms.

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