IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF PREGABALIN AND DULOXETINE HCL IN BULK AND PHARMACEUTICAL DOSAGE FORM BY UV AND RP-HPLC USING HUMAN PLASMA

[1]R.N.Sathyaprakash , [2]S.Ravichandran
Department of Pharmaceutical Analysis, PSG College of Pharmacy
Tamil Nadu, India.

Abstract: This work is concerned with a new, simple, rapid, and precise UV and RP-HPLC method for determination of pregabalin and duloxetine hydrochloride in both bulk and in capsule. UV determination was carried out using Acetonitrile: water as solvent system. The maximum absorbance was observed at 267nm and 218nm for pregabalin and duloxetine hydrochloride respectively. Chromatographic separation was achieved isocratically using C18 column (250×4.6 mm, 5µm), mobile phase containing Acetonitrile and phosphate buffer (pH 6.8) in the ratio 40:60 maintained at flow rate 1 ml/min. Detection wavelength at 240nm. Retention time of pregabalin was found to be 1.8min and for duloxetine HCl 3.4 min. This method is then used for analysis of formulation. The developed method was validated. The validation of proposed method was verified by recovery studies and was found to be satisfactory.

Keywords: UV, RP-HPLC, pregabalin, duloxetine hydrochloride, method development, validation

INTRODUCTION

Pregabalin is structurally similar to gamma aminobutyric acid, an inhibitory neurotransmitter. It is used to treat neuropathic pain, posttherapic neuralgia and fibromyalgia. IUPAC name is (3S)-3-(aminomethyl)-5-methylhexanoic acid.

Duloxetine hydrochloride is a selective SSNRI. It is mainly used to treat anxiety disorder and diabetic peripheral neuropathy. IUPAC name is N-methyl-3-napthalen-1-oxy-3-thiophen-2-yl-amine.

An extensive survey on literature reveals that HPLC and UV method for the simultaneous estimation of pregabalin and duloxetine hydrochloride combination has not yet been reported.

Fig1: structure of pregabalin

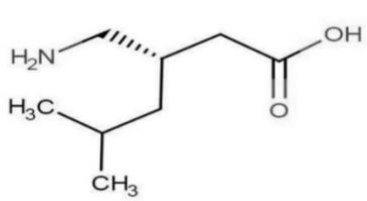
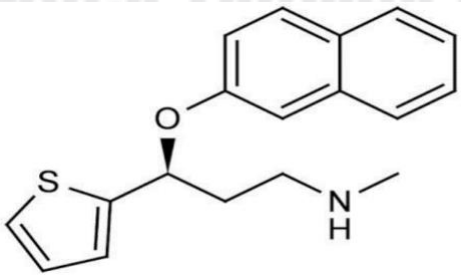


Fig2: structure of duloxetine hydrochloride



Chemicals and Reagents:

Working Standard samples of pregabalin and Duloxetine hydrochloride are obtained and Capsule was procured from local pharmacy, with labelled amount containing 50 mg and 20 mg of pregabalin and duloxetine hydrochloride respectively. Acetonitrile

(HPLC Grade) was obtained from Loba Chemie Pvt Ltd, Potassium dihydrogen phosphate and Disodium hydrogen phosphate obtained from Thermo Fischer Pvt Ltd.

UV Method Development:

Selection of solvent:

For both pregabalin and duloxetine HCl, the absorbance was found to be good in Acetonitrile : water (40:60). The maximum absorbance was found to be 267nm and 218nm for pregabalin and duloxetine HCL respectively.

Preparation of stock solution:

10 mg of pregabalin and duloxetine HCl was weighed and transferred to volumetric flask and made upto the volume with solvent system having concentration 1000µg/ml. Calibration curve was plotted by serial dilutions of pregabalin having concentration 2-10 µg/ml. similarly, Calibration curve was plotted for duloxetine HCl having concentration 1-5 µg/ml.

Preparation of sample solution:

The capsule of formulation was emptied and weighed accurately powder equivalent to 50mg of pregabalin and 20 mg of duloxetine HCl was weighed and diluted with solvent system. The absorbance of sample solution was then measured and the concentration was estimated by simultaneous equation method.

Simultaneous equation method:

The wavelength selected for the analysis of formulation are 267nm and 218nm respectively. The concentration of two drugs in the mixture was calculated using the formula,

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

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

Where,

Cx, Cy are the concentration of pregabalin and duloxetine hydrochloride respectively,

A1, A2 are the absorbance of formulation at 267nm and 218nm respectively,

ax1, ax2 are the absorptivities of pregabalin at 267nm and 218nm respectively,

ay1, ay2 are the absorptivities of duloxetine hydrochloride at 267nm and 218nm respectively.

Absorptivity = absorbance / concentration

Table 1: Absorptivity value for pregabalin

Concentration (µg/ml)	λ 1 – 267nm		λ 2 – 218nm	
	Absorbance	Absorptivity	Absorbance	Absorptivity
2	0.221	0.1105	0.068	0.034
4	0.413	0.1032	0.074	0.0185
6	0.605	0.1008	0.078	0.013
8	0.765	0.0956	0.081	0.081
10	0.970	0.097	0.096	0.0096
	ax1 = 0.10143		ax2 = 0.031	

Table 2: Absorptivity value for duloxetine hydrochloride

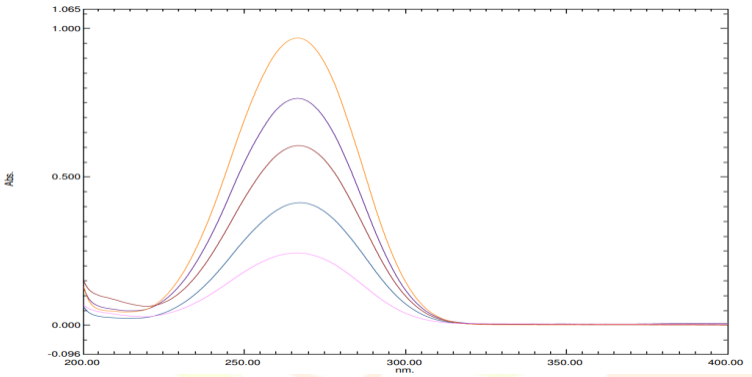
Concentration (µg/ml)	λ 1 -267nm		λ 2 -218nm	
	Absorbance	Absorptivity	Absorbance	Absorptivity
1	0.019	0.019	0.165	0.165
2	0.044	0.022	0.345	0.172
3	0.070	0.023	0.536	0.178
4	0.109	0.027	0.751	0.187
5	0.195	0.039	0.927	0.185
	ax1 = 0.02606		ax2 = 0.17748	

Table 3: Analysis of marketed formulation

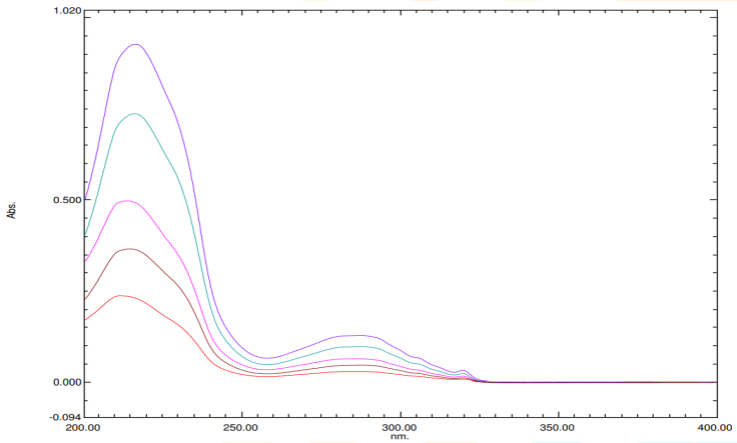
S.No	DRUG	AMOUNT		% AMOUNT FOUND
		LABELLED	FOUND	
1	Pregabalin	50	49.75	99%
2	Duloxetine HCl	20	19.75	98%

Calibration curve:

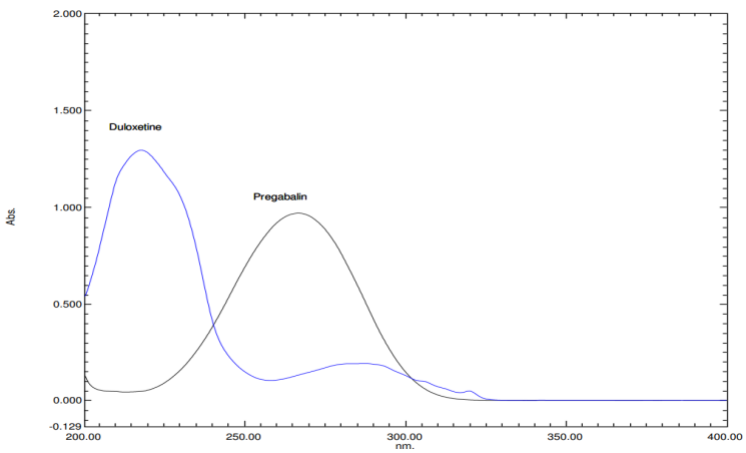
The calibration curve was plotted over a concentration range of 2-10 µg/ml for pregabalin and 1-5 µg/ml for duloxetine hydrochloride.



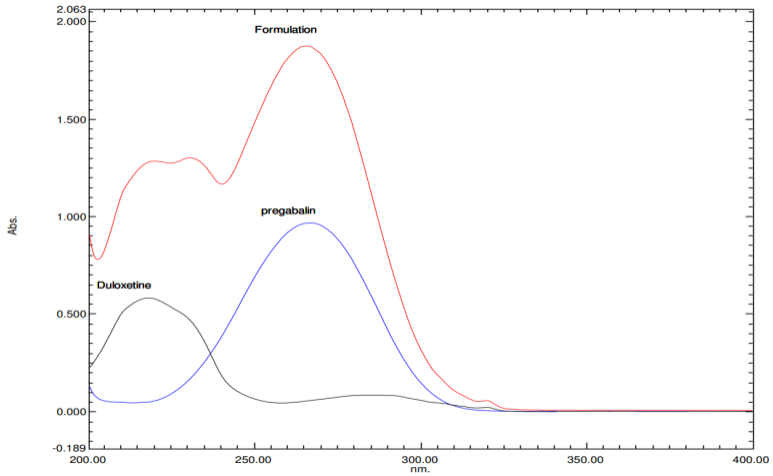
Overlain spectra of pregabalin



Overlain spectra of Duloxetine hydrochloride



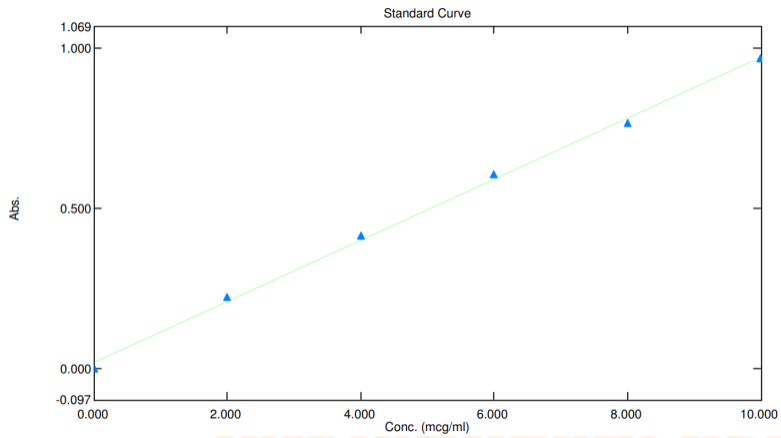
Overlay of pregabalin and duloxetine hydrochloride (isobestic point)



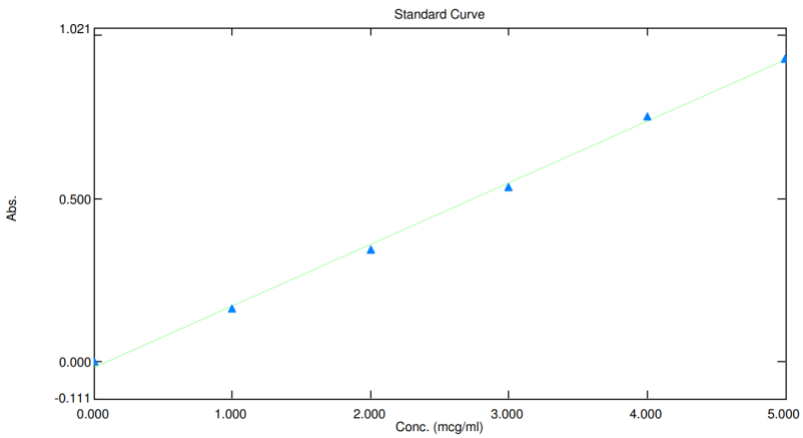
Overlain spectra of standard and formulation

METHOD VALIDATION

1. linearity: Pregabalin was found to be linear at the concentration range of 2-10 mcg/ml. Duloxetine hydrochloride was found to be linear at the concentration range of 1-5 mcg/ml. The absorbance was noted at both wavelength 267nm and 218nm, calibration curve was plotted using concentration vs absorbance. The correlation coefficient (r^2) was found to be 0.998.



Linearity graph of pregabalin at 267nm



Linearity graph of Duloxetine HCl at 218nm

2. precision: Precision should be measured using minimum three determination per concentrations. In intraday and interday variation study, nine different solution of concentration i.e.,(6, 8, 10 ppm of pregabalin and 3, 4, 5 ppm of duloxetine hydrochloride), were analysed. From the absorbance result mean, standard deviation and %RSD were calculated and given in following table,

Table 4: Interday precision

Sl.no	Concentration (µg/ml)		Absorbance				%RSD			
	PGB	DUL	PGB (267nm)	DUL (218nm)	PGB (267nm)	DUL (218nm)	PGB	DUL	PGB	DUL
1	6	3	0.605	0.236	0.080	0.535	0.165	0.244	0.727	0.186
			0.606	0.237	0.079	0.536				
			0.607	0.236	0.079	0.537				
2	8	4	0.765	0.294	0.080	0.752	0.130	0.340	1.234	0.132
			0.765	0.295	0.082	0.753				
			0.766	0.293	0.081	0.751				
3	10	6	0.970	0.353	0.086	0.927	0.102	0.283	1.162	0.062
			0.971	0.353	0.087	0.927				
			0.970	0.352	0.087	0.928				

Table 5: Intraday precision

Sl.no	Concentration (µg/ml)		Absorbance				%RSD			
	PGB	DUL	PGB (267nm)	DUL (218nm)	PGB (267nm)	DUL (218nm)	PGB	DUL	PGB	DUL
1	6	3	0.605	0.236	0.080	0.535	0.095	0.244	0.737	0.107
			0.606	0.237	0.079	0.536				
			0.607	0.236	0.079	0.537				
2	8	4	0.765	0.294	0.080	0.752	0.075	0.341	0.709	0.076
			0.765	0.295	0.082	0.753				
			0.766	0.293	0.081	0.751				
3	10	6	0.970	0.353	0.086	0.927	0.059	0.163	0.666	0.001
			0.971	0.353	0.087	0.927				
			0.970	0.352	0.087	0.928				

Table 6: Repeatability of pregabalin

concentration	Absorbance	
	267nm	218nm
10 µg/ml	0.970	0.106
	0.969	0.107
	0.970	0.106
	0.971	0.105
	0.970	0.107
	0.970	0.106
Mean	0.970	0.106
SD	0.00063	0.00075
%RSD	0.064	0.707

Table 7: Repeatability of duloxetine hydrochloride

concentration	Absorbance	
	267nm	218nm
5 µg/ml	0.353	0.927
	0.352	0.928
	0.353	0.927
	0.354	0.926
	0.353	0.927
	0.353	0.928
Mean	0.353	0.927
SD	0.00063	0.00075
%RSD	0.707	0.08

3. limit of detection & limit of quantitation:

The LOD and LOQ of the drugs were calculated with standard deviation and their slope values.

LOD = 3.3 × SD/ SLOPE

LOQ = 10 × SD/ SLOPE

Table 8: LOD and LOQ

Drugs	Parameter			
	LOD		LOQ	
	267nm	218nm	267nm	218nm
PGB	0.5	0.15	1.57	0.46
DUL	0.18	0.23	0.56	0.71

RP-HPLC METHOD

1. Selection of chromatographic method:

The selection of the method depends upon the nature of the sample, its molecular weight and solubility. The drug selected in the study is polar and RP-HPLC method was preferred due to its suitability.

2. Selection of wavelength: From the UV studies, the isobestic point 240nm was selected for HPLC analysis.

3. Optimization of mobile phase: Optimization of mobile phase was done by trial and error method. Different mobile phase like methanol : water, Acetonitrile : water was used. Finally water is replaced with phosphate Buffer pH 6.8 and trail was carried out.

Chromatographic condition:

Chromatographic separation was achieved isocratically using C18 column (250 × 4.6 mm), injection volume 20 µl. mobile phase consisted of acetonitrile :phosphate buffer (40:60) maintained at flow rate 1ml/min. Detection done using PDA detector at 240nm.

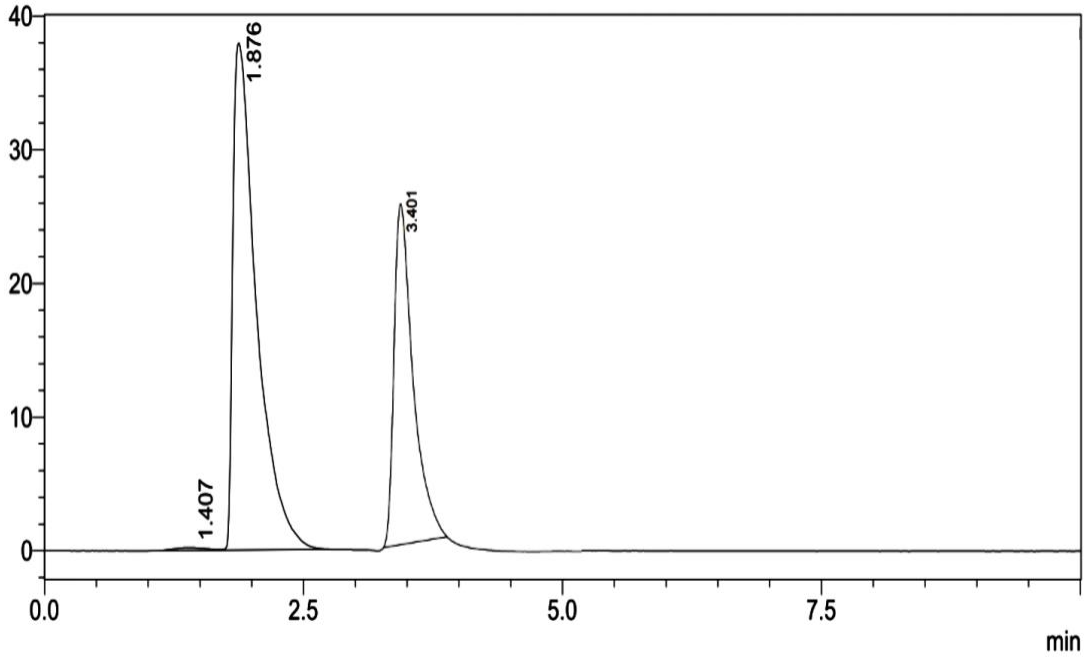
Preparation of mobile phase

The phosphate buffer solution was prepared by dissolving 13.872g of potassium dihydrogen phosphate and 35.084g of disodium hydrogen phosphate in sufficient water to produce 1000 ml.

Preparation of pregabalin standard solution:

Weighed about 10 mg pregabalin and transferred into 10 ml volumetric flask and made upto volume with mobile phase. From the stock solution, a series of dilutions are made to obtain concentration of 5, 10, 15, 20 and 25 µg/ml and used for HPLC analysis.

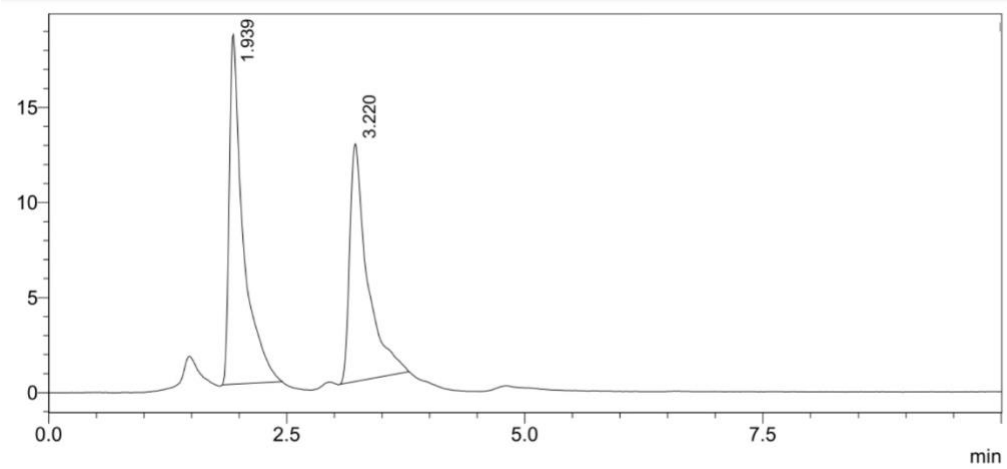
Preparation of duloxetine HCl standard solution: Weighed about 10 mg duloxetine HCl and transferred into 10 ml volumetric flask and made upto the volume with mobile phase. From the stock solution, a series of dilutions aare made to obtain concentration 2, 4, 6, 8, 10 µg/ml and used for HPLC analysis



Chromatogram of standard

Analysis of formulation:

The formulation was analysed by dissolving the drug in capsule using mobile phase, filtered using Whatmann filter paper. The stock solution was then diluted to obtain concentration equivalent to 50/20 µg/ml in 10 ml mobile phase. The formulation was then injected with same chromatographic condition.



Chromatogram of formulation

BIO-ANALYTICAL METHOD:

FIXED EXTRACTION PROCEDURE

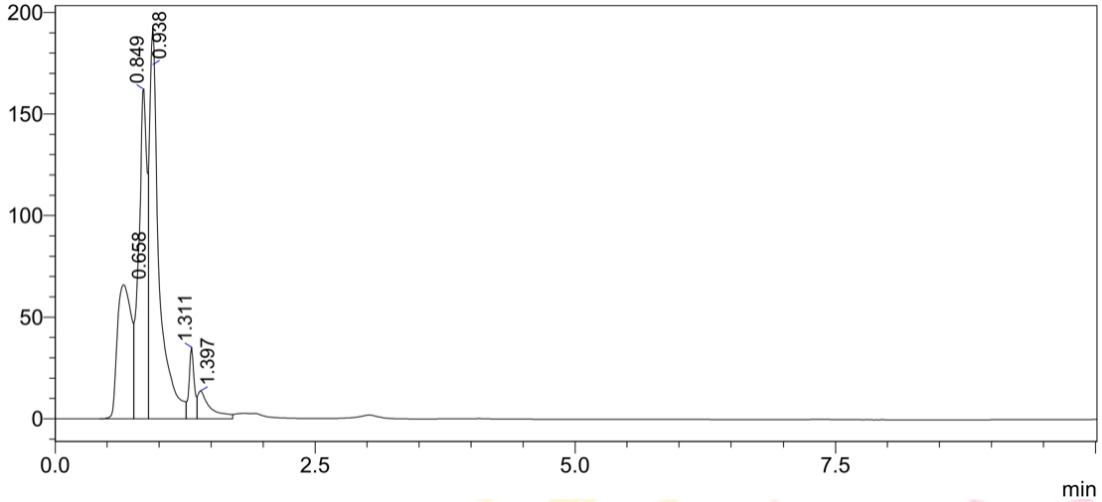
0.5 ml of pregabalin 2-10 mcg/ml and duloxetine HCl 1-5 mcg/ml were pipetted out and spiked into 0.5 ml plasma in separate polypropylene tube (Eppendorf). Then the tubes was kept in vortex mix for 5 minutes. Then 1 ml of acetonitrile was added to the tubes and centrifuged for 20 minutes at 3000 rpm. Further supernatant liquids were collected in another Eppendorf tube and supernatant was injected into analytical column.

ANALYSIS OF FORMULATION

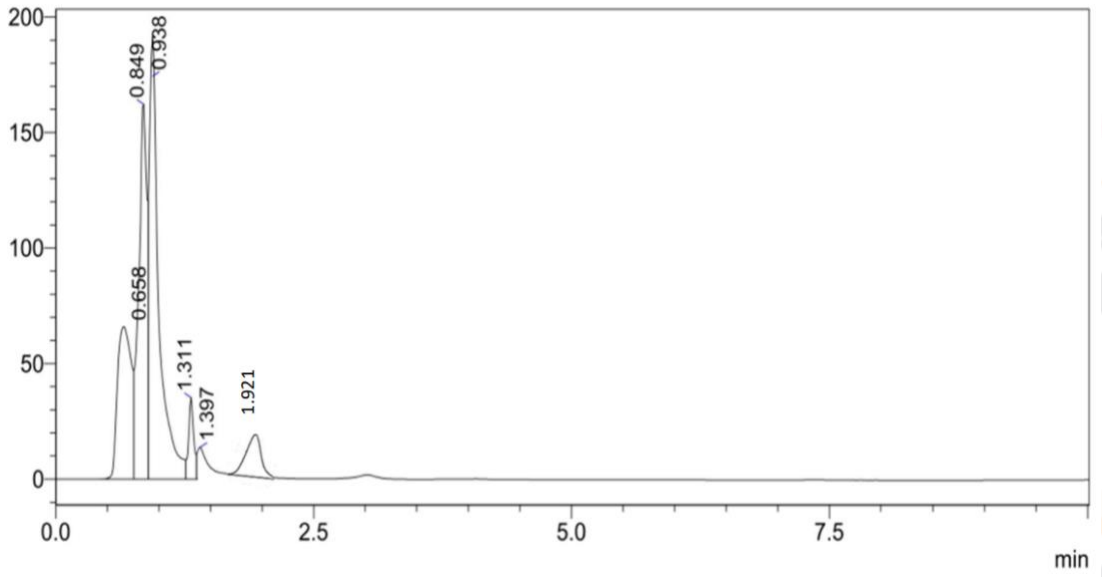
The Capsule containing 50 mg of pregabalin and 20 mg of duloxetine hydrochloride was emptied and weighed accurately the powder equivalent to 10 mg of pregabalin and 10 mg of duloxetine hydrochloride. The weighed powder was transferred into a clean, dry 100ml volumetric flask. The powder was dissolved in sufficient volume of mobile phase by sonication. the resulting suspension was then filtered through whatman filter paper. The volume of filtrate was made up to 100ml with mobile phase and Further diluted, having concentration of 100µg/ml. 0.5 ml of sample is taken in a eppendrof tube containing 0.5 ml plasma and kept in vortex for 5 mins. To this 1 ml of acetonitrile is added and centrifuged for 20 mins at 3000 rpm. Further supernatant liquids were collected in another eppendrof tube and supernatant was injected into analytical column.

Chromatogram of blank plasma

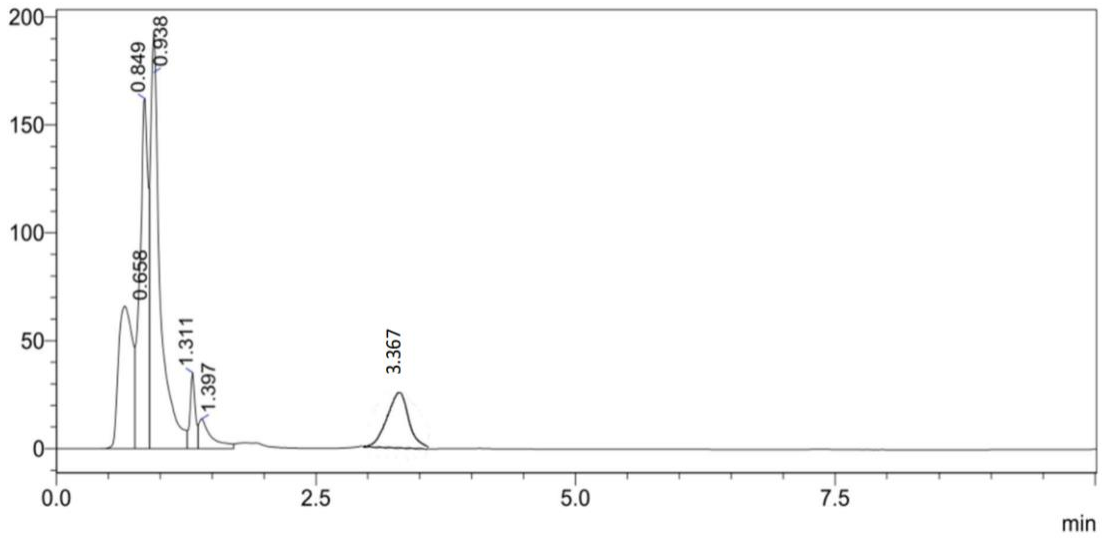
Blank solution was prepared and chromatogramed. It was found that there was some interference from blank plasma.



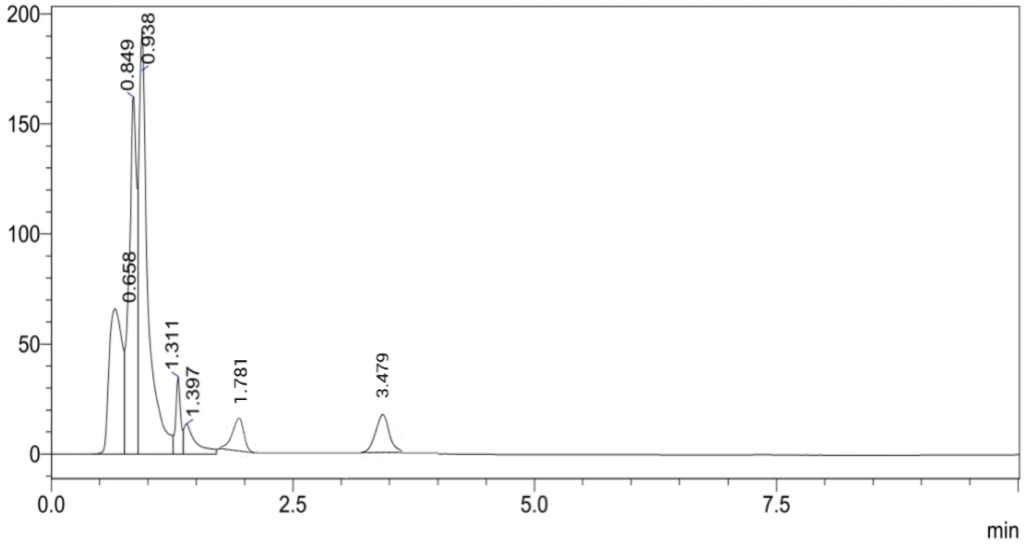
Chromatogram of plasma



Chromatogram of pregabalin in plasma



Chromatogram of duloxetine HCl in plasma



Chromatogram of formulation in plasma

VALIDATION OF METHOD

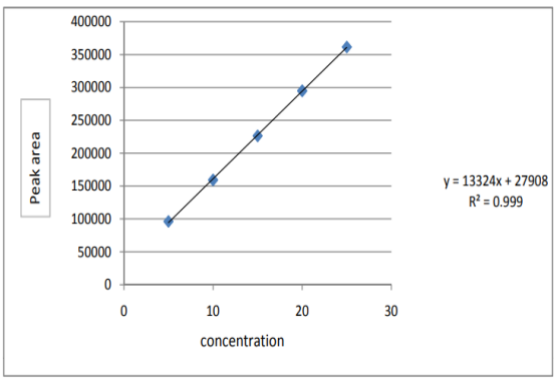
1. Specificity

From the blank chromatogram, mixture of standard drug solution and sample reveal that the peaks observed are due to the presence of drug. This confirms the specificity of the method developed.

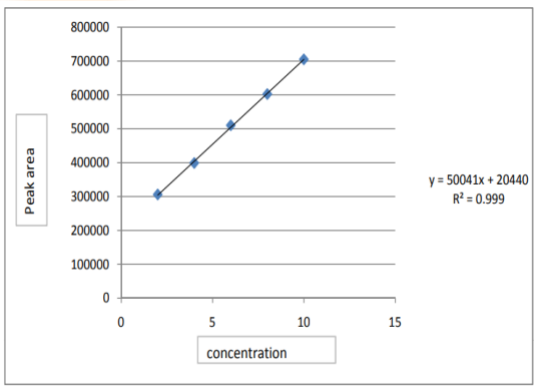
2. Linearity

A calibration curve is a relationship between the instrument response and a known concentration of the analyte. It was observed that the optimized method was linear by constructing a graph using peak area vs concentration. The concentration was found to be linear in the range from 5-25 µg/ml of pregabalin and 2-10 µg/ml of duloxetine hydrochloride. The correlation coefficient (r^2) was found to be 0.999 for both pregabalin and duloxetine hydrochloride. From the graph equation for the straight line was obtained along with the slope and y-intercept values.

Linearity of Duloxetine HCL



linearity of Pregablin



3.Precision

Precision of the method was demonstrated by interday, intraday, repeatability studies. Intraday precision was found by carrying out the analysis at three different concentrations in linearity range for three times on the same day. Interday precision was found by carrying out the analysis at three different concentration in linearity range for three days over a period of one week. Repeatability was done by injecting five times the same concentration and %RSD was determined and tabulated.

Table 9: Interday Precision

Sl.no	Concentration		Peak area		%RSD	
	PGB	DUL	PGB	DUL	PGB	DUL
1	5	2	306270	96607	1.75	0.51
			306380	97400		
			316197	97521		
2	10	4	399096	159442	1.59	0.14
			399345	159492		
			399227	159507		
3	15	6	510362	226469	1.3	0.502
			510556	226621		
			510227	226333		

Table 10: Intraday Precision

Sl.no	Concentration		Peak area		%RSD	
	PGB	DUL	PGB	DUL	PGB	DUL
1	5	2	317270	96532	1.915	1.14
			306380	96400		
			305197	98541		
2	10	4	399176	152242	1.55	1.56
			398345	154792		
			409599	149987		
3	15	6	513362	238469	1.30	1.97
			500654	232771		
			510277	229333		

Table 11: Repeatability

No. of injections	Concentration		Peak area		% RSD	
	PGB	DUL	PGB	DUL	PGB	DUL
1	15	6	519962	229245	1.35	1.79
2			520066	236789		
3			510345	226012		
4			520517	226469		
5			519619	232312		
6			520720	225961		

4. Accuracy

The accuracy of the optimized method was determined by absolute and relative recovery. It was found that replicating analysis of sample containing a known amount of analyte. Based on the calibration curve, the accuracy of pregabalin and duloxetine HCl was found using three percentage of 80%, 100%, 120%. Based on the peak area/ height % recovery was calculated.

Table 12: Accuracy studies

Sl. No	Spiked level	Recovery		% RSD	
		PGB	DUL	PGB	DUL
1	80%	99.5%	99.5%	0.05	0.06
2	100%	101.6%	101.8%		
3	120%	100.2%	100.1%		

5. LOD and LOQ:

The lowest amount of analyte in a sample that can be detected under stated experimental conditions and the Limit of quantification is the lowest amount of analyte in the sample that is quantified and is usually established by injecting the linearity concentration of standard solution at which the peak was determined. The limit of detection and limit of quantification was calculated by using the average value of standard deviation and slope.

Table 13: LOD and LOQ

Drugs	LOD	LOQ
PGB	0.41	1.26
DUL	0.89	2.12

6. Robustness:

The robustness of the method was studied by changing the method like alteration in flow rate, pH, changes in the wavelength. It was observed that there are no changes in the chromatograms demonstrating that the HPLC methods have developed are robust.

Table 14: Robustness

Parameters	Changes done	Peak area	
		PGB	DUL
Flow rate	0.8 ml	510066	221498
	1 ml	527389	226784
	1.2 ml	532271	228431
Detection wavelength	238nm	522127	224491
	240nm	527389	226784
	242nm	528512	228497
Mobile phase ratio	58:42	514916	224794
	60:40	527389	226784
	62:38	529712	234671

7. System Suitability:

System suitability of the method was performed by calculating the chromatographic parameters like Column efficiency (theoretical plates), Resolution factor, Peak asymmetric factor, tailing factor were measured and calculated by repetitive injection using the system suitability test solution. These tests are carried out based on the concept that the equipment, analytical method and samples are an integral part of the system that can be evaluated. Then coefficient of variation for peak area response and retention time for the test substance was determined. System suitability was not more than 2% RSD for pregabalin and duloxetine hydrochloride. The results obtained are within the acceptance criteria.

Parameter	PGB	DUL
Retention time	1.8 min	3.4 min
Peak area	550912	229214
Tailing factor	1.89	1.51
No. of theoretical plate	5172	1749

SUMMARY AND CONCLUSION:

In this study, pregabalin and duloxetine hydrochloride in combined dosage form was estimated by the proposed UV, RP-HPLC method. The fixed-dose combination capsule of pregabalin and duloxetine hydrochloride was subjected to simultaneous estimation by UV spectroscopic method.

A new, accurate, simple and precise RP-HPLC method for simultaneous identification and quantification of pregabalin and duloxetine hydrochloride in pharmaceutical dosage form with PDA detection has been developed and validated.

The developed method was used for the estimation of drug in bulk and its formulation in human plasma by in-vitro method. Acetonitrile was used as extracting solvent.

Reference:

1. Haritha Potluri, Sreenivasa Rao Battula ,Sunandamma Yeturu, Validated Stability Indicating Rp-Hplc Method For Simultaneous Determination Of Nortriptyline And Pregabalin In Bulk And Combined Dosage Formulations. Journal Of Chilean Chemical Society. Jan 2017.

2. B.Sivagami, S.Kavya Lalitha, V.Pavan Kumar, R.Sireesha, R.Chandrasekar. Development And Validation of Stability Indicating Rp-Hplc Method for The Simultaneous Estimation of Pregabalin and Epalrestat in Tablet Dosage Forms. International Journal of Pharmacy.2018,9(5).

3. J.Anil Mohan, B.Rajkumar, T.Bhavya, A.Ashok Kumar. 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 Science, Vol 6 , 2014.

4. N.Kannapan, S.P.Nayak, T.Venkatachalam, V.Prabhakaran. Analytical Rp-Hplc Method for Development and Validation of Pregabalin and Methylcobalamine in Combined Capsule Formulation. Journal Of Applied Chemical Research,13,85-89(2010)

5. Ashu M, Parmar S, Nagarajan K, Vijendra Singh. Development And Validation of Rapid Hplc Method for Determination of Pregabalin in Bulk and Capsule Dosage Forms. Der Pharma Chemical, 2011,3(1):482-489

6. Seema A, Jeeja P, Ashish J. Development and Validation of Hplc Method for The Estimation of Pregabalin in Bulk and Capsule Dosage Forms. Pharmaceutica Analytica Acta, 2016.

7. Chusena Narasimharaju Bhimanadhuni, Devala Rao Garikapati, Chintha Srinivas. Development And Validation of Rp-Hplc Method for Determination of Duloxetine Hydrochloride in Bulk and Dosage Forms. International Current Pharmaceutical Journal 2012,1(5):98-102.

8. Usmangani K, Chhalotiya, Kashyap K. Bhatt, Dimal A.Shah, Sunil L. Baldania. Development And Validation of a Stability Indicating Rp-Hplc Method for Duloxetine Hydrochloride in Its Bulk and Tablet Dosage Form. Sci Pharm.2010;78:857-868.

9. D.Boopathy, Rahul Dadaroji Jawarkar, M.Prakash, Bibin Mathew And P.Perumal. New Rp-Hplc Method Development and Validation for Estimation of Duloxetine Hcl in Enteric Coated Capsules. International Journal of Chemtech Research, Vol 2, No.1, Pp 239-241. Jan-Mar 2010.

10. M.M. Kamila , N.Mondal, L.K.Ghosh. A Validated UV Spectrophotometric Method For Determination Of Duloxetine Hydrochloride. Pharmazine 62: 414-415 (2007).

11. Mohammad Yunoos, D.Gowri Sankar, B.Pragati Kumar, Shahul Hameed, Azmath Hussain. Simple Uv Spectrophotometric Determination Of Duloxetine Hydrochloride In Bulk And In Pharmaceutical Formulations. E-Journal Of Chemistry 2010,7(3), 785-788.

12. Jogi Naga Kumari, Development And Validation Of New Rp-Hplc Method For Simultaneous Estimation Of Drug Duloxetine And Mecobalamin In Tablet Dosage Forms Indian Journal Of Research In Pharmacy And Biotechnology, Issn: 2321-5674.

13. Chhaya K. Chauhan, Development And Validation Of Analytical Method For Simultaneous Estimation Of Duloxetine And Methylcobalamin In Pharmaceutical Dosage Form, International Journal Of Advances In Pharmaceutical Sciences, Vol 6, March-April 2015, Pg 2815-2817

14. Jampala Balaji, Bandi Ramachandra, N.V.S.Naidu, Analytical RP-HPLC method for development and Validation of pregabalin in bulk and the determination of pregabalin in capsule dosage form, International Journal Of Pharmaceutical Research in Science, Engineering and Technology, Vol 3, Issue 4, April 2014.

15. Swapna Goday, Abdul Rahaman, Prameelarani A, Development of a new stability indicating RP-HPLC method for simultaneous estimation of Epalrestat and pregabalin and its validation as per ICH Guidelines, Asian Journal of pharmaceutical and clinical science, Vol 11, issue 5, 2018.

16. P. Sowndarya, Md. Naseeruddin, Syed Ghouse, A. Ashok Kumar, Assay method development and validation for simultaneous estimation of duloxetine and methylcobalamin in capsules by RP-HPLC, Der Pharmacia Lettre, 8 (7):52-60, 2016.

17. Prasanna A Datar, Rohan U Waghmare, Development and validation of an analytical method for the stability of duloxetine hydrochloride. Journal of Taibah University for Science**,** Volume 8, Issue 4, October 2014, Pages 357-363