IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 3, March 2026
Impact Factor: 8.2

A Validated RP-HPLC Method for Simultaneous Determination of Pregabalin and Duloxetine in Pharmaceutical Dosage Forms

Ms. Rutuja Ganpat Dhakne[1*] and Dr. K Sarvanan[2]
Research Scholar, Bhagwant Global University, Kotdwar, Uttarakhand[1]
Research Guide, Bhagwant Global University, Kotdwar, Uttarakhand[2]

Abstract: ***Background:*** *A simple, rapid, and reliable reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Pregabalin and Duloxetine in bulk drug and pharmaceutical dosage forms. Chromatographic separation was achieved using a C18 column (4.6 × 100 mm, 2.5 µm particle size) with a mobile phase consisting of methanol and 0.1% orthophosphoric acid buffer (75:25 v/v). The flow rate was maintained at 0.8 mL/min, and detection was carried out at 215 nm using a UV detector. Under optimized chromatographic conditions, the retention times of Pregabalin and Duloxetine were found to be 3.02 min and 6.50 min, respectively, with good peak resolution and symmetrical peak shapes. The developed method was validated according to the guidelines of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use with respect to system suitability, accuracy, precision, and robustness. The percentage assay for Pregabalin and Duloxetine in bulk drugs was found to be 99.97% and 100.86%, respectively, indicating excellent agreement with the labeled claim. Recovery studies at different concentration levels showed percentage recoveries close to 100%, confirming the accuracy of the method. Precision studies revealed low %RSD values, demonstrating good reproducibility. The robustness study indicated that small variations in chromatographic conditions did not significantly affect the analytical results. The developed RP-HPLC method was found to be accurate, precise, and reliable and can be successfully applied for routine quality control analysis of Pregabalin and Duloxetine in pharmaceutical formulations.*

Keywords: Pregabalin, Duloxetine, RP-HPLC, Method Development, Method Validation, Simultaneous Estimation, Pharmaceutical Analysis, ICH Guidelines

I. INTRODUCTION

Neuropathic pain and major depressive disorders are among the most common neurological and psychiatric conditions affecting millions of individuals worldwide. These disorders significantly reduce the quality of life and often require long-term pharmacological management. Combination therapy involving antiepileptic and antidepressant agents has gained considerable attention in recent years due to their synergistic therapeutic effects in the management of neuropathic pain, fibromyalgia, and generalized anxiety disorders. Among such combinations, Pregabalin and Duloxetine are widely prescribed owing to their proven efficacy and safety profile.

Pregabalin is a structural analogue of gamma-aminobutyric acid (GABA) and acts by binding to the α2-δ subunit of voltage-gated calcium channels in the central nervous system, thereby reducing the release of excitatory neurotransmitters such as glutamate, norepinephrine, and substance P. Due to this mechanism, Pregabalin is widely used for the treatment of neuropathic pain, epilepsy, fibromyalgia, and generalized anxiety disorder. Duloxetine, on the other hand, is a serotonin-norepinephrine reuptake inhibitor (SNRI) that increases the levels of serotonin and norepinephrine in the synaptic cleft, enhancing inhibitory pain pathways in the central nervous system. It is commonly prescribed for major depressive disorder, diabetic neuropathic pain, fibromyalgia, and chronic musculoskeletal pain.

DOI: 10.48175/IJARSCT-31606

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The combined use of Pregabalin and Duloxetine has been reported to provide enhanced therapeutic benefits in the treatment of neuropathic pain and related disorders. Pharmaceutical formulations containing these two drugs require reliable analytical techniques for accurate quantification to ensure product quality, safety, and regulatory compliance. High-performance liquid chromatography (HPLC) is one of the most widely used analytical techniques for the simultaneous estimation of drugs in pharmaceutical dosage forms due to its high sensitivity, accuracy, and reproducibility.

Several analytical methods have been reported for the individual determination of Pregabalin and Duloxetine; however, only a limited number of methods describe their simultaneous estimation in combined dosage forms. Moreover, many reported methods involve complex mobile phase compositions, longer retention times, or gradient elution systems that may not be suitable for routine quality control laboratories. Therefore, there is a need to develop a simple, rapid, and reliable analytical method with good resolution and shorter analysis time.

In the present study, an attempt was made to develop and validate a simple reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous determination of Pregabalin and Duloxetine in pharmaceutical dosage forms. The developed method was optimized to achieve efficient chromatographic separation with good peak symmetry and resolution within a short run time. The method was further validated according to the guidelines of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use with respect to system suitability, accuracy, precision, and robustness. The validated method is expected to be suitable for routine quality control analysis of these drugs in pharmaceutical formulations.

II. MATERIALS AND METHOD

The simultaneous estimation of Pregabalin and Duloxetine was carried out using a reverse-phase high-performance liquid chromatography (RP-HPLC) method. Pure drug samples of Pregabalin and Duloxetine were obtained as reference standards, while marketed pharmaceutical formulations containing both drugs were procured from a local pharmacy. All solvents and reagents used in the study were of HPLC grade, including methanol and orthophosphoric acid used for preparation of the buffer solution. The chromatographic analysis was performed using an Agilent Technologies gradient HPLC system equipped with an auto-injector and UV diode array detector. Separation was achieved on a Fortis C18 column (Cosmosil) (4.6 × 100 mm, 2.5 µm particle size). The mobile phase consisted of methanol and 0.1% orthophosphoric acid buffer in the ratio of 75:25 (v/v), which was filtered through a membrane filter and degassed prior to use. The flow rate of the mobile phase was maintained at 0.8 mL/min, and detection was carried out at a wavelength of 215 nm under ambient temperature conditions. The injection volume was 20 µL, and chromatographic data were recorded and processed using Chemstation 10.1 software. Standard stock solutions of both drugs were prepared by accurately weighing appropriate quantities of Pregabalin and Duloxetine and dissolving them in the mobile phase, followed by suitable dilution to obtain working concentrations. For analysis of the marketed formulation, tablets were accurately weighed, finely powdered, and an amount equivalent to the labeled drug content was transferred into a volumetric flask, dissolved in the mobile phase with sonication, filtered, and diluted appropriately. The developed method was validated according to the guidelines of the **International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) for parameters including system suitability, accuracy, precision, robustness, and assay to ensure the reliability and reproducibility of the analytical method.

III. RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions

The RP-HPLC method for the simultaneous determination of **Pregabalin** and **Duloxetine** was optimized by evaluating different chromatographic parameters such as mobile phase composition, flow rate, and detection wavelength. Several combinations of organic solvents and buffer systems were tested to obtain well-resolved and symmetrical peaks. Optimal separation with good peak shape and minimal tailing was achieved using a **mobile phase consisting of**

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methanol and 0.1% orthophosphoric acid buffer (75:25 v/v). The analysis was performed on a **C18 column (4.6 × 100 mm, 2.5 µm particle size)** with a flow rate of **0.8 mL/min** and detection at **215 nm** using a UV detector. Under these optimized conditions, both drugs showed well-resolved chromatographic peaks with acceptable retention times and good baseline separation.

System Suitability

System suitability parameters were evaluated to ensure the efficiency and reliability of the chromatographic system. The retention times for Pregabalin and Duloxetine were found to be **3.02 ± 0.022 min** and **6.50 ± 0.01 min**, respectively. The theoretical plate count was **8203 for Pregabalin** and **9746 for Duloxetine**, indicating good column efficiency. The tailing factors were found to be close to unity (**1.00 for Pregabalin and 1.11 for Duloxetine**), suggesting symmetrical peak shapes. The resolution value between the two peaks was **2.34**, confirming adequate separation of the two analytes. These parameters demonstrate that the developed chromatographic method is suitable for the simultaneous estimation of both drugs.

Assay of Bulk Drug

The developed RP-HPLC method was applied for the quantitative determination of Pregabalin and Duloxetine in bulk drug samples. The percentage assay results were **99.97% for Pregabalin** and **100.86% for Duloxetine**, with very low %RSD values (**0.33% and 0.23%, respectively**). These results indicate excellent accuracy and reproducibility of the developed method for the analysis of both drugs in bulk form.

Analysis of Marketed Formulation

The validated RP-HPLC method was successfully applied for the analysis of marketed pharmaceutical formulations containing Pregabalin and Duloxetine. The percentage drug content for Pregabalin was found to be **99.81 ± 0.56%**, with a %RSD of **1.64%**, indicating good agreement with the labeled claim. Similarly, Duloxetine showed a mean drug content of **99.82 ± 0.28%** with a %RSD of **1.82%**. These results demonstrate that the developed method is reliable and suitable for routine quality control analysis of pharmaceutical formulations containing these drugs.

Accuracy

Accuracy of the method was evaluated using recovery studies at three levels (80%, 100%, and 120%) by the standard addition method. For Pregabalin, the percentage recoveries ranged from **99.16% to 101.07%**, with a %RSD below **0.33%**. For Duloxetine, the recovery values ranged between **99.33% and 100.70%**, with %RSD values below **0.37%**. The recovery values close to 100% indicate that the developed method is highly accurate and free from interference from formulation excipients.

Precision

Precision of the analytical method was evaluated in terms of **interday and intraday precision**. For Pregabalin, the %RSD values were **0.33% (interday)** and **0.38% (intraday)**, whereas for Duloxetine the %RSD values were **0.51% (interday)** and **0.70% (intraday)**. The low %RSD values obtained for both drugs indicate good repeatability and reproducibility of the method.

Robustness

Robustness of the method was assessed by introducing small deliberate variations in chromatographic conditions such as flow rate, mobile phase composition, wavelength, and temperature. The %RSD values obtained under these varied conditions were found to be less than **2%** for both Pregabalin and Duloxetine. These results indicate that minor changes in experimental conditions do not significantly affect the chromatographic performance, demonstrating the robustness of the developed RP-HPLC method.

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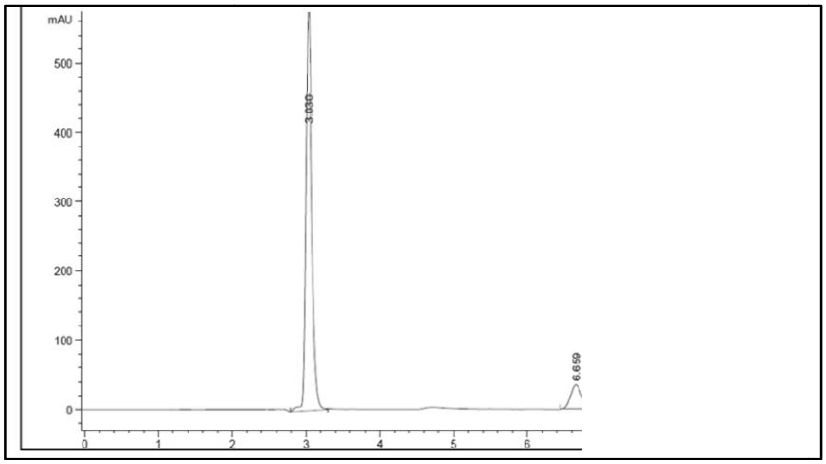
Optimization of a solvent system



Optimized chromatogram of Pregabalin and Duloxetine

Chromatographic conditions

Chromatographic Mode	Chromatographic Conditions
HPLC System	Agilent Tech. Gradient system with Auto injector
Detector	UV (DAD)
Column	fortis C18 (Cosmosil)(4.6×100µm with 2.5µm particle size)
Mobile phase	Methanol : Buffer solution(0.1% OPA) (75:25v/v)
Detection wavelength	215 nm
Flow rate	0.8mL/min
Temperature	Ambient
Injection Volume	20 µL
Data analysis	Chemstation 10.1

System suitability test/parameters

Parameters	Estimates for Pregabalin	Estimates for Duloxetine
Retention time (Rt) (min)	3.02 ± 0.022	6.50 ± 0.01
Theoretical Plates	8203 ± 0.56	9746 ± 0.11
Tailing factor	1.00 ± 0.02	1.11 ± 0.03
Resolution	2.34	

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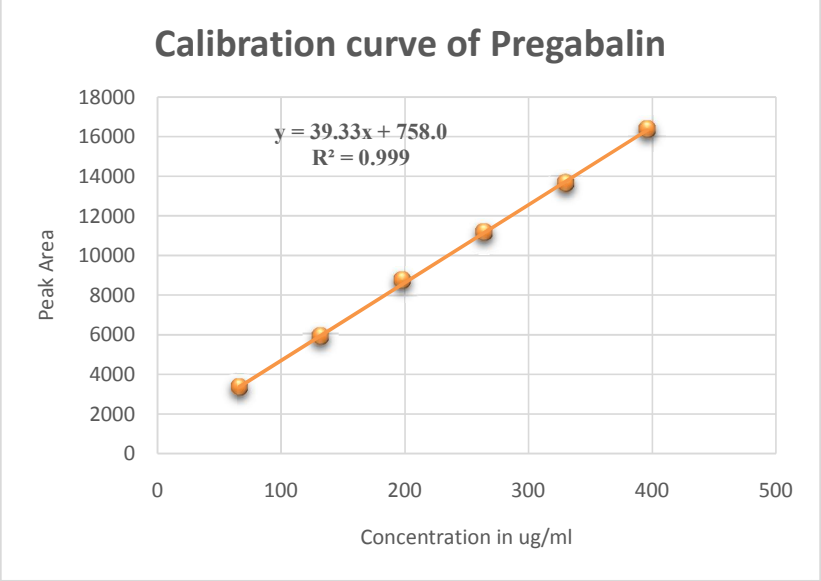

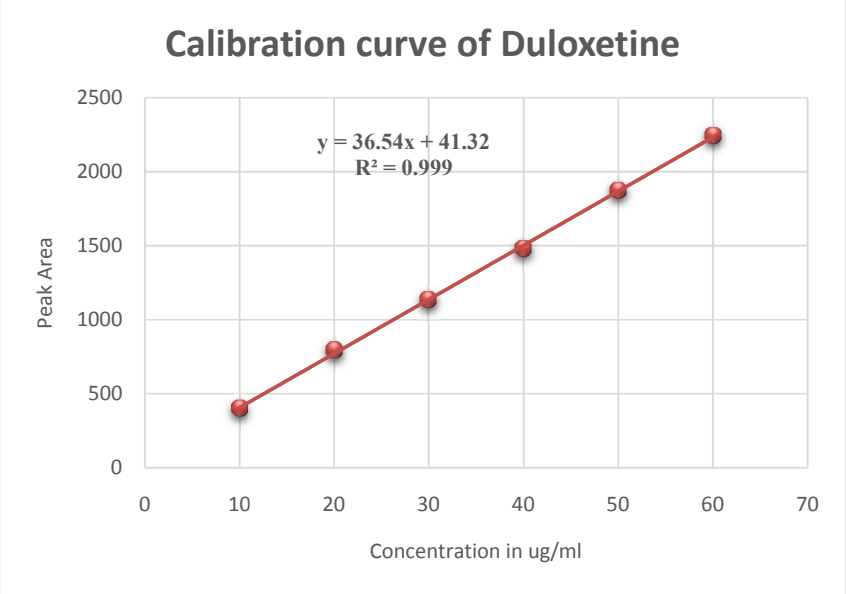

Analysis (Assay) of Pregabalin and Duloxetine in bulk material

Drugs	Amount taken [µg/mL]	Amount found [µg/mL] ± SD	% Amount found	% RSD {n=6}
Pregabalin	66	65.97 ± 0.05	99.97 ± 0.57	0.33
Duloxetine	10	10.26 ± 0.32	100.86 ± 0.16	0.23

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Analysis of marketed formulation

Drug name	Conc. 50 ppm; Peak Area	Amount found[µg/mL]	% Amount found
Pregabalin	4153577	48.80	97.61
	4182566	49.14	98.29
	4238045	49.80	99.60
	4288000	50.38	100.77
	4308597	50.62	101.25
	4312570	50.67	101.35
	Mean ± STD. DEV.	**49.90± 0.12**	**99.81± 0.56**
	RSD (%)	**1.64**	**1.64**

Drug name	Conc. 100 ppm; Peak Area	Amount found[µg/mL]	% Amount found
Duloxetinein	11791974	98.61	98.61
	11817679	99.29	99.29
	11836991	99.60	99.60
	11918728	100.17	100.17
	11940675	100.55	100.55
	11962649	100.75	100.75
	Mean ± STD. DEV.	**99.82± 0.28**	**99.82± 0.28**
	RSD (%)	**1.82**	**1.82**

Accuracy data of Pregabalin and Duloxetine

Pregabalin					
Sr.No.	**Accuracy level**	**Initial Conc.**	**Amount spiked**	**Amount recover % Recovery**	**Statistical Analysis**
1	**80%**	66	52.8	99.78	Mean=99.46 SD=0.31 %RSD=0.31
2		66	52.8	99.16	
3		66	52.8	99.43	
1	**100%**	66	66	101.07	Mean=100.81 SD=0.34 %RSD=0.33
2		66	66	100.43	
3		66	66	100.93	
1	**120%**	66	79.2	100.63	Mean=100.64 SD=0.17 %RSD=0.17
2		66	79.2	100.81	
3		66	79.2	100.47	
Duloxetine					
Sr.No.	**Accuracy level**	**Initial Conc.**	**Amount spiked**	**Amount recover % Recovery**	**Statistical Analysis**
1	**80%**	10	8	99.33	Mean= 99.59 SD= 0.23 %RSD=0.23
2		10	8	99.77	
3		10	8	99.66	
1	**100%**	10	10	100.70	Mean=100.46 SD=0.32 %RSD=0.31
2		10	10	100.57	
3		10	10	100.10	

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1	**120%**	10	12	99.51	Mean=99.91 SD=0.37 %RSD=0.37
2		10	12	99.98	
3		10	12	100.25	

Precision data of Pregabalin

Pregabalin				
Sr. No.	**Conc.**	**Peak area**	**Amount found % Assay**	**Statastical analysis**
Interday				
1	132	5997.93	100.88	SD= 4.21 %RSD= 0.33
2	198	8764.80	102.89	
3	264	11195.75	100.65	
Intraday				
1	132	5976.94	100.48	SD= 8.24 %RSD= 0.38
2	198	8570.80	100.39	
3	264	11128.45	100.00	

Duloxetine				
Sr. No.	**Conc.**	**Peak area**	**Amount found % Assay**	**Statastical analysis**
Interday				
1	20	791.18	102.24	SD= 5.64 %RSD= 0.51
2	30	1136.26	99.98	
3	40	1486.05	99.03	
Intraday				
1	20	785.04	101.68	SD= 7.45 %RSD= 0.70
2	30	1129.44	99.36	
3	40	1487.75	99.15	

Robustness data of Pregabalin and Duloxetine

Sr. No.	**Condition/Parameters**	**Mean Peak Area ± SD %RSD for Pregabalin**	**Mean Peak Area ± SD %RSD for Duloxetine**
1	Flow rate(-) 0.7ml/min	0.29	0.71
2	Flow rate(+) 0.9ml/min	0.05	0.16
3	Mobile Phase (-) 74+26 v/v	0.16	0.52
4	Mobile Phase (+) 76+24 v/v	0.12	0.07
5	Wavelength (-) 218nm	0.38	0.71
6	Wavelength (+) 222nm	0.20	0.70

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7	Temperature 30˚C	0.28	0.19
8	Temperature 40˚C	0.37	0.23

IV. CONCLUSION

A simple, sensitive, and reliable RP-HPLC method was successfully developed for the simultaneous determination of Pregabalin and Duloxetine in bulk and pharmaceutical dosage forms. The optimized chromatographic conditions provided good resolution, symmetrical peak shapes, and acceptable retention times for both analytes. Validation studies demonstrated that the proposed method is accurate, precise, robust, and reproducible, with recovery values close to 100% and low %RSD values. The assay results for the marketed formulation were within acceptable limits, confirming the suitability of the method for pharmaceutical analysis. Therefore, the developed RP-HPLC method can be effectively applied for routine quality control and quantitative estimation of Pregabalin and Duloxetine in pharmaceutical preparations in research laboratories and pharmaceutical industries.

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