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DEVELOPMENT AND VALIDATION OF NOVEL RP- HPLC METHOD FOR SIMULTANEOUS ANALYSIS OF PREGABALIN AND DULOXETINE IN SYNTHETIC MIXTURE

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ABSTRACT: A sensitive, rapid, precise and accurate RP-HPLC method was developed for simultaneous estimation of Pregabalin and Duloxetine in synthetic mixture. To introduce a chromophoric group in Pregabalin structure, derivatization was carried out through benzoylation reaction. RP- HPLC method is carried out using Hypersil BDS column C_{18} (250 cm × 4.6 cm, 5 µm). Mobile phase used as combination of Methanol: Water (75:25 v/v) and detection was carried out at 235 nm & 224 nm respectively for Duloxetine and Pregabalin. Retention time of Duloxetine and Pregabalin were found to be 2.15 min and 5.23 min, respectively. The developed method was validated in pursuance of ICH Q2 (R1) guidelines. Linearity range of Duloxetine is 50-100 µg/mL and for Pregabalin is 100-200 µg/mL. Correlation coefficient for both drugs was 0.999 and mean recoveries for both drugs were between 99% - 100%, minimum values of %RSD indicate the accuracy of the method. The detailed quantitative results show that this method is sensitive, precise as well as cost effective.

INTRODUCTION: Duloxetine (DULO) is a potent dual reuptake inhibitor of serotonin and nor epinephrine used to treat major depressive disorders [1-2]. Chemical name of DULO is 3- (amino methyl)-5-methylhexanoic acid. It has no significant affinity for dopaminergic, adrenergic, cholinergic, histaminergic, opioid, glutamate, and GABA receptors. Pregabalin (PREGA), an antiepileptic drug similar to gabapentin produces its actions by binding to the alpha2-delta (α2δ) subunit of the voltage-gated calcium channels [1, 3].

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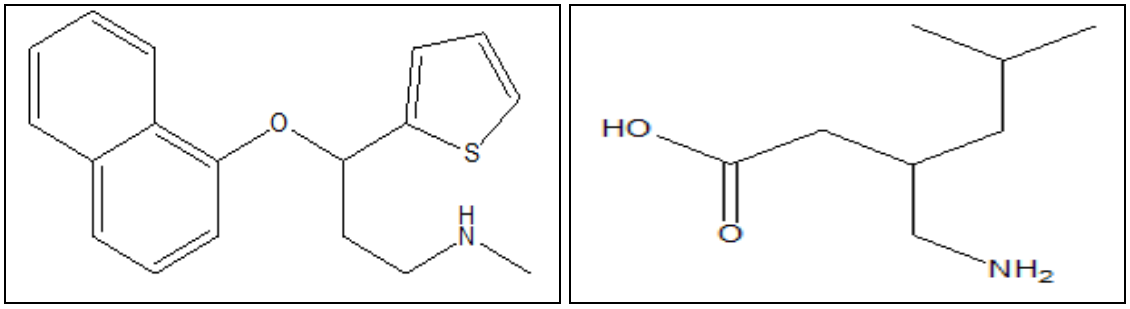
Chemical name of PREGA is N- methyl-3-naphthalen - 1 – yloxy - 3 - thiophen - 2 -ylpropan-1-amine. Respective structure of DULO and PREGA is shown in **Fig. 1.** The ratio of DULO and PREGA in Synthetic mixture is 1: 2, respectively. DULO is official in USP and PREGA is official in IP. Various pharmacopoeial [4] and reported methods like UV [5-6]; RP- HPLC [7-11] has been developed for the estimation of PREGA. While pharmacopoeial[12] and RP-HPLC [13-14] and HPTLC [15] methods are reported for estimation of DULO.

This research paper describes the development and validation of novel RP- HPLC method for simultaneous estimation of DULO and PREGA in their synthetic powder mixture. This analytical method is optimized, developed and validated as per ICH Q2 (R1) guidelines [16].



(A) Duloxetine **(B) Pregabalin**

FIG. 1: STRUCTURES OF A) DULO AND B) PREGA

Derivatization of Pregabalin through Benzoylation [17-18]: Pregabalin (3- (amino methyl)-5-methylhexanoic acid) does not have chromophoric group. For UV analysis, it is compulsory to have a chromophoric group in the structure. To introduce a chromophoric group in pregabalin structure, convert primary amino group of Pregabalin through reaction with Benzoyl chloride to form benzoylated derivative of pregabalin. It shows maximum absorbance at 224 nm using UV-visible spectrophotometer. Benzoyl chloride is Chromogenic agent. This Method is applicable and suitable for routine quality control of Pregabalin API and pharmaceutical formulation without any interference of excipients.

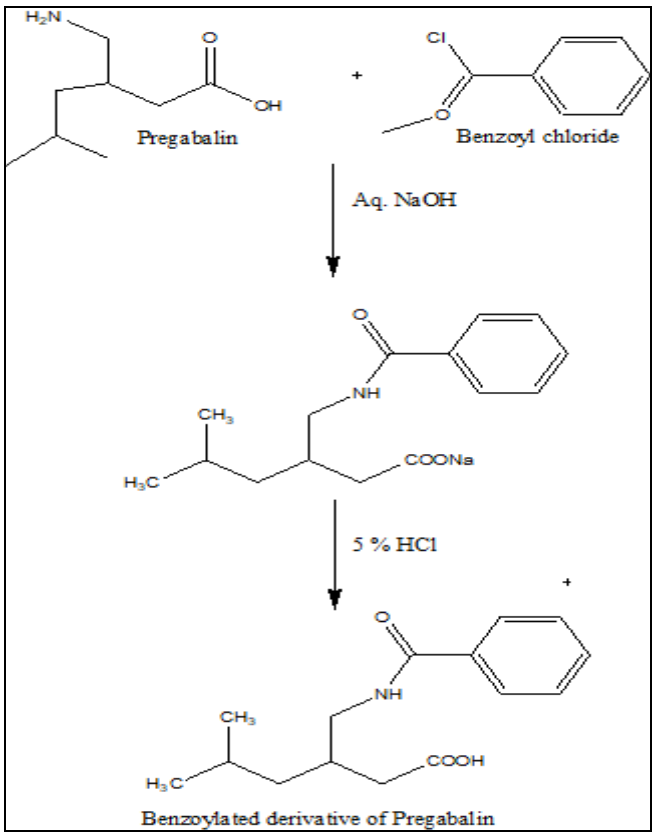


FIG. 2: SCHEME FOR DERIVATIZATION OF PREGABALIN THROUGH BENZOYLATION

MATERIALS AND METHODS:

Instrumentation: A Shimadzu LC-10 AT HPLC instrument was used for chromatographic separation. This HPLC system consists of UV detector having 20µL fixed loop.

Chromatogram was recorded and peaks were qualified by software Delta Ace station. Chromatographic separation was carried out on a C_{18} Hypersil BDS column having 25 cm × 0.46 cm, 5 µm diameters.

Reagents and Chemicals: API of Pregabalin and Duloxetine procured as gift samples from Sunrise remedies, Santej. Water, Methanol and Acetonitrile, was HPLC grade purchased from Finar Ltd. Potassium di-hydrogen orthophosphate, o- phosphoric acid was Analytical grade purchased from Ranbaxy chemicals.

Chromatographic Conditions: Optimized mobile phase consisting Water: Methanol in ratio 25:75 was used in an isocratic mode. Reagents was initially filtered through 0.45 micron membrane filter and then sonicated for 15 minutes before used as mobile phase. Flow rate was maintained at 1.0 mL/min and injection volume was 20 µL.

Method Development of RP- HPLC Method:

Selection of Wavelength: The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drug that are to be detected.

In the present study drug solutions of Duloxetine (0.5 mg/mL) and Pregabalin (1.0 mg/mL) was prepared in Methanol. This drug solution was than scanned in UV region of 200-400 nm and maximum Absorbance was recorded.

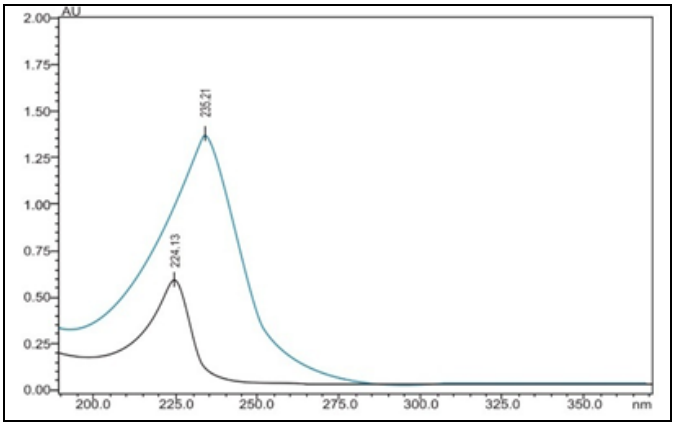


FIG. 3: UV SPECTRA OF DULOXETINE (235 NM) AND PREGABALIN (224 NM)

Preparation of Stock and Working Solution: Take accurately weighed 5 mg of DULO and 10 mg of PREGA. It is separately transferred into 10 mL volumetric flasks. DULO (volumetric flask A) and PREGA (volumetric flask B) was dissolved using methanol and make up the volume upto with methanol. These stock solutions have 0.5 mg/mL and 1.0 mg/mL for DULO and PREGA, respectively. From the volumetric flask A, 1 mL was transferred into 10 mL vol. flask and volume was made with methanol to get final dilution of 50 µg/mL of DULO. Then from volumetric flask B, 1 mL was transferred into 10 mL vol. flask and volume was made with methanol to get final dilution of 100 µg/mL of PREGA.

System Suitability Parameter: System suitability was carried out by injecting 100% concentration into chromatographic system. These are repeated six times under similar condition. The system suitability parameters like Theoretical Plates per column (N), Tailing factor (T), Resolution (Rs) and Retention time were studied and found satisfactory. Obtained results are shown in **Table 1.** Optimized Chromatogram of DULO and PREGA is shown in **Fig. 4.**

TABLE 1: SYSTEM SUITABILITY PARAMETERS

Parameters	DULO	PREGA
Retention Time (Min)	2.982	6.689
Theoretical plates per column (N)	7843	10564
Symmetry factor/Tailing factor (T)	0.99	1.01
Resolution (Rs)	1.989	

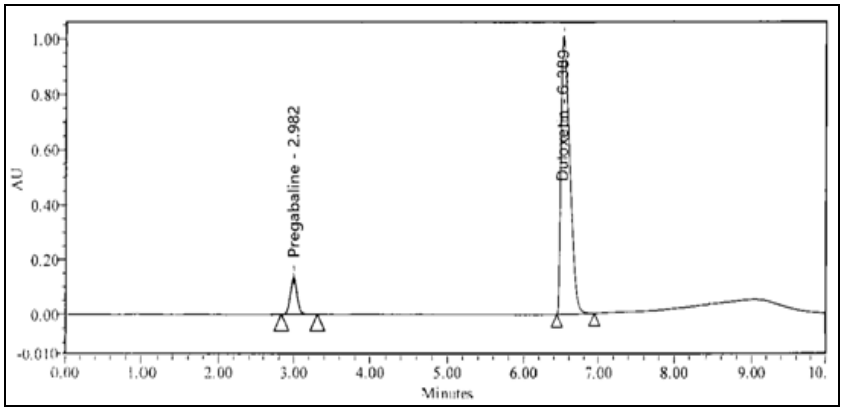


FIG. 4: OPTIMIZED FINAL CHROMATOGRAM OF DULO AND PREGA

Method Validation of RP- HPLC Method:

Linearity and Range: The linearity for Duloxetine and Pregabalin was assessed by analysis of combined standard solution in range of 50-100 µg/mL and 100- 200 µg/mL for Duloxetine and Pregabalin, respectively.Standard Calibration curve was plotted and Correlation coefficient (r^2) was found.

System Precision (Repeatability): Repeatability was carried out by injecting six replicates of 75 µg/mL Duloxetine and 150 µg/mL Pregabalin. Then average peak area and % RSD were calculated.

Intermediate Precision: The Intermediate Precision of analytical method demonstrated by Intraday and Interday Precision.

Intraday Precision: In Intraday precision, Standard solution containing Duloxetine(62.5, 75, 87.5 µg/mL) and pregabalin (125, 150, 175 µg/mL) were analyzed three times on the same day (0 hour, 3 hours and 6 hours) and then average peak area and % RSD were calculated.

Interday Precision: In Interday precision, Standard solution containing Duloxetine(62.5, 75, 87.5 µg/mL) and pregabalin (125, 150, 175 µg/mL)

were analyzed three times on the different day (Day-1, 2, 3) and then average peak area and % RSD were calculated.

Accuracy (Recovery Study): To measure accuracy of analytical method, recovery studies were carried out using standard addition method with different level 80 %, 100 % and 120 %. The results of recovery studies indicated that the method is accurate for the estimation of Duloxetine and Pregabalin.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): Limit of Detection is a lowest concentration in a sample that can be detected but not necessarily quantified under the optimized experimental conditions. The Limit of Quantitation is lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy.

Robustness: The robustness of method was determined under variable conditions. The robustness of developed analytical method was established by illustrating its reality against consider changes in the optimized chromatographic conditions.

Assay: The proposed RP-HPLC method was successfully applied for simultaneous determination of Duloxetine and Pregabalin in the synthetic powder mixture.

RESULT AND DISCUSSION:

Linearity and Range: The linearity for Duloxetine and Pregabalin was assessed by analysis of combined standard solution in range of 50-100 µg/mL and 100- 200 µg/mL for Duloxetine and Pregabalin, respectively. The regression line equation for Duloxetine and Pregabalin is as following: For Duloxetine y = 36,495.016x - 18, 08, 009.20, Pregabalin y = 21,551.368x - 21, 30, 393.60.

The correlation coefficient (r^2) was found to be 0.998 of both the drug. Linearity data of DULO and PREGA are described in **Table 2**, respectively. The calibration curves for DULO and PREGA are shown in **Fig. 5A and 5B**. Overlay Chromatogram for DULO and PREGA is also shown in **Fig. 5C.**

TABLE 2: LINEARITY DATA FOR DULOXETINE AND PREGABALIN

Sr. no.	Concentration (µg/mL)		Peak Area (n=5) ± S.D.		% R.S.D.	
	DULO	PREGA	DULO	PREGA	DULO	PREGA
1	50	100	165873 ± 77.88	215634 ± 100.16	0.046	0.046
2	62.5	125	322087 ± 61.40	418712 ± 118.19	0.019	0.028
3	75	150	626313 ± 108.85	814207 ± 110.86	0..017	0.013
4	87.5	175	1290914 ± 116.47	1678188 ± 105.63	0.009	0.006
5	100	200	1911398 ± 185.63	2484817 ± 103.23	0.009	0.004

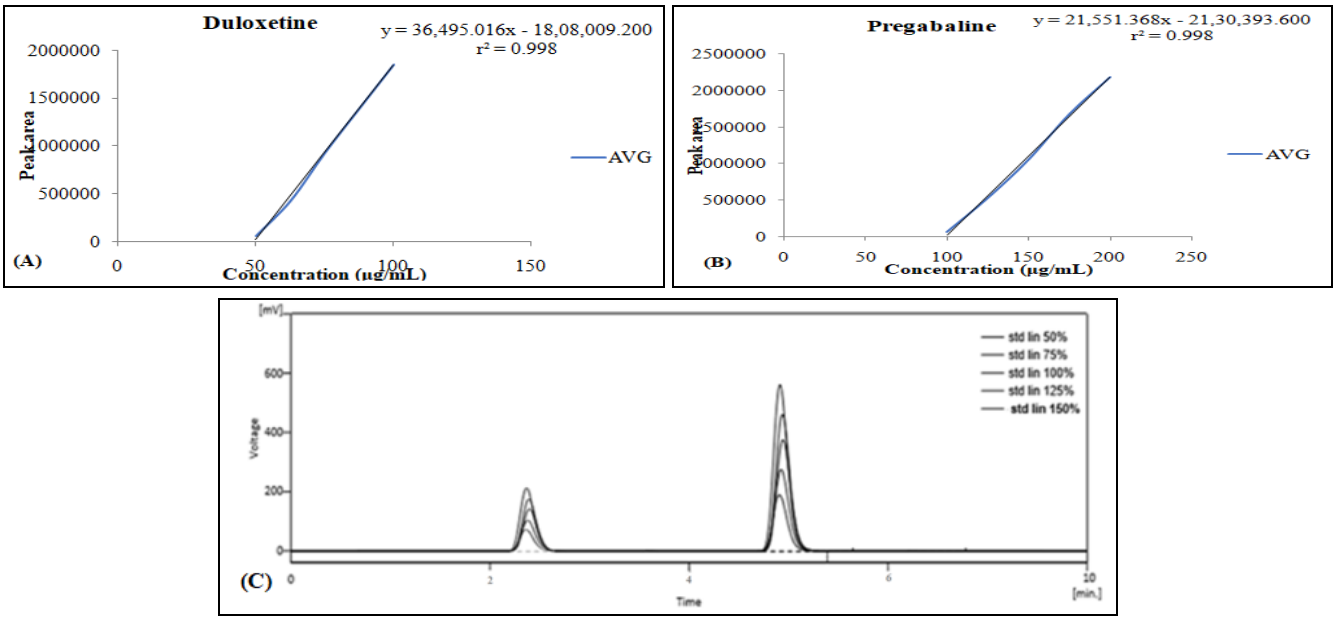


FIG. 5: (A) CALIBRATION CURVE OF DULO; (B) CALIBRATION CURVE OF PREGA; (C) LINEARITY OVERLAY SPECTRA OF DULO AND PREGA

System Precision (Repeatability): The data for repeatability of peak area measurement for Duloxetine and Pregabalin; based on six measurements of same solution of Duloxetine (75 µg/mL) and Pregabalin (150 µg/mL) is depicted in **Table 3 and 4**. The % RSD for Duloxetine and Pregabalin were found to be 0.106 and 0.048, respectively.

TABLE 3: REPEATABILITY DATA FOR DULOXETINE

Sr. no.	Conc. (µg/mL)	Area	Mean ± S.D (n=6)	% R.S.D
1.	75	625413		0.106
		625959	625744.33 ± 66.51	
		626058		
		626395		
		626087		
		624554		

TABLE 4: REPEATABILITY DATA FOR PREGABALIN

Sr. no.	Conc. (µg/mL)	Area	Mean ± S.D (n=6)	% R.S.D
1.	150	814207	814354.53 ± 39.62	0.048
		814632		
		814924		
		813804		
		814434		
		814125		

Intermediate Precision: The Intermediate precision of analytical method demonstrated by Intraday and Interday Precision.

Intraday Precision: In Intraday Precision, %RSD in Intraday precision for Duloxetine and Pregabalin were found in range of 0.050- 0.092 and 0.055-0.115, respectively.

TABLE 5: INTRADAY PRECISION DATA FOR ESTIMATION OF DULOXETINE AND PREGABALIN

Sr. no.	Conc. (µg/mL)		Area Mean ± S.D. (n=3)		% R.S.D	
	DULO	PREGA	DULO	PREGA	DULO	PREGA
1	62.5	125	322009.33 ± 28.32	418296.33 ±48.24	0.088	0.115
2	75	150	626345.42 ± 31.70	814146 ± 55.51	0.050	0.068
3	87.5	175	1288556 ± 119.2	1677345 ± 93.74	0.092	0.055

Interday Precision: In Interday Precision, % RSD in Interday precision for Duloxetine and Pregabalin were found in range of 0.091- 0.102 and 0.094-0.139, respectively.

TABLE 6: INTERDAY PRECISION DATA FOR ESTIMATION OF DULOXETINE AND PREGABALIN

Sr. no.	Conc. (µg/mL)		Area Mean ± S.D. (n=3)		%R.S.D	
	DULO	PREGA	DULO	PREGA	DULO	PREGA
1	62.5	125	322045.33 ± 33.14	418296.13 ± 58.22	0.102	0.139
2	75	150	626444.66 ± 57.45	814049.33 ± 64.20	0.091	0.078
3	87.5	175	1288622.66 ±129.10	1682911.66 ± 159.7	0.100	0.094

Accuracy (Recovery Study): The amount of DULO and PREGA was calculated and % recovery found satisfactory.

TABLE 7: RECOVERY DATA FOR DULOXETINE (N=3)

Sr. no.	Conc. Level (%)	Sample amount (µg/mL)	Amount Added (µg/mL)	Amount Recovered (µg/mL)	% Recovery	% RSD
1	80 %	75	60	59.44	99.72	0.46
2	100 %	75	75	74.83	99.88	0.41
3	120 %	75	90	89.99	99.99	0.53

TABLE 8: RECOVERY DATA FOR PREGABALIN (N=3)

Sr. no.	Conc. Level (%)	Sample amount (µg/mL)	Amount Added (µg/mL)	Amount recovered (µg/mL)	% Recovery	% RSD

1	80 %	150	120	119.67	99.87	1.14
2	100 %	150	150	149.79	99.92	0.62
3	120 %	150	180	180.02	100.00	0.44

LOD and LOQ: Results of LOD and LOQ were given in **Table 9.**

TABLE 9: LOD AND LOQ DATA FOR DULOXETINE AND PREGABALIN

Duloxetine	
LOD	**LOQ**
LOD = 3.3 x (SD / Slope) = 3.3 x (727468.54 /12,75,945) =2.05 µg/mL	LOQ = 10 x (SD / Slope) = 10x (727468.54 /12,75,945) =5.70 µg/mL
Pregabalin	
LOD	**LOQ**
LOD = 3.3 x (SD / Slope) = 3.3 x (945709.34/ 16,58,728.92) = 1.88 µg/mL	LOQ = 10 x (SD / Slope) = 10x (945709.34/ 16,58,728.92) = 5.70 µg/mL

Robustness: Developed HPLC Method is found to be robust as the results were not significantly affected by slight variation in composition of Mobile phase and Flow rate. Values obtained of % RSD are given in **Table 10.**

TABLE 10: ROBUSTNESS DATA

Condition	**Variation**	**Pregabalin**		**Duloxetine**	
		Area (n=3) ± SD	**% RSD**	**Area (n=3) ±SD**	**% RSD**
Flow rate (± 0.1)	1.1 mL/min	626365.66 ±151.96	0.024	814316 ±143.87	0.017
	0.9 mL/min	626332.33 ±102.91	0.016	814349.33 ± 89.07	0.010
Composition of Mobile phase (± 2)	+2 mL	626362.33 ±86.55	0.013	814345.66± 85.51	0.010
	-2 mL	626397.66 ± 150.18	0.024	814409 ± 106.57	0.013

Assay of Synthetic Mixture: The proposed RP-HPLC method was successfully applied for simultaneous determination of Duloxetine and Pregabalin in the synthetic mixture. The results are shown in **Table 11.**

TABLE 11: RESULTS OF ASSAY (N=3)

Sr. no.	**Sample**	**Label claim (mg)**	**Area of Sample**	**Result (mg)**	**Avg. % Assay ± S.D.**	**% RSD**
1.	Duloxetine	0.5	9556990	0.499	99.96 ± 0.05	0.054
2.	Pregabalin	1	24848170	0.998	99.91 ± 0.05	0.052

CONCLUSION: A Simple, economic, rapid and selective RP- HPLC method has been optimized, developed and validated for simultaneous estimation of Duloxetine and Pregabalin in Synthetic powder mixture. The method fulfilled the requirements to be considered a realizable method, including all validation parameters- Specificity, Linearity, Precision, accuracy, robustness, LOD and LOQ. All method validation parameters are complies with its acceptance criteria as per ICH Q2 (R1) guideline. So we concluded that method is selective, accurate, linear and precise also. Hence, it can also successfully used for the routine analysis.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

REFERENCES:

1. Rang HP and Dale MM: Rang and Dale's Pharmacology. Churchill Livingstone Elsevier limited, New York, Sixth Edition 2007; 557-558.
2. Satoskar RS, Bhandarka SD and Rege NN: Pharmacology and Pharmacotherapeutics. Popular Prakashan Private Limited, Mumbai, Twenty fifth Edition 2010; 203.
3. Goyal RK: Elements of Pharmacology. B. S. Shah Prakashan, Ahmedabad, Ninteenth Edition 2009; 10: 309-310.
4. Indian Pharmacopoeia, Government of India Ministry of Health and Family welfare, Volume- III, The Indian Pharmacopeia Commission, Ghaziabad 2014; 2548-2549.
5. Gujral R, Haque M and Shanker P: Development and Validation of Pregabalin in Bulk, Pharmaceutical

Formulations and in Human Urine Samples by UV Spectrophotometry. Int J Biomed Sci 2009; 5(2): 175–180.
6. Chhajed SS, Sonawane S, Pingle A and Dashputre N: Development and validation of UV spectrophotometric method for estimation of pregabalin. Asian J Pharm Ana 2019; 9(1): 15-18.
7. Kasawar GB and Farooqui MN: Development and Validation of HPLC Method for the determination of Pregabalin in Capsules. Indian J Pharm Sci 2010; 72(4): 517–519.
8. Kannapan N, Nayak SP, Venkatachalam T and Prabhakaran V: Analytical RP-HPLC method for development and validation of pregabalin and methylcobalamine in combined capsule formulation. J Applied Chem Res, 2010; 13(1): 85- 89.
9. Balaji J, Ramachandra B and Naidu NVS: Analytical RP-HPLC Method for Development and Validation of Pregabalin in Bulk and the Determination of Pregabalin in Capsule dosage form. Int J Inno Res Sci Eng Techno 2014; 3(4): 11094- 11098.
10. Pingale P and Singasane T: Development and validation of HPLC method for the determination of pregabalin in bulk and in pharmaceutical formulations. Res J Pharm Techno 2012; 5(6): 829-833.
11. Ponnekanthi K, Sunitha K and Ganapaty S: Development and Validation of new RP-HPLC Method for simultaneous Estimation of Methylcobalamin, Epalrestat and Pregabalin in bulk and Pharmaceutical dosage form. Res J Pharm Techno 2021; 14(10): 5097-6000.
12. United state Pharmacopoeia, Second Supplement, USP 35–NF 30 2012; 5939-5940.
13. Chhalotiya UK, Bhatt KK, Shah DA and Baldania SL: 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.
14. Datar PA and Waghmare RU: Development and validation of an analytical method for the stability of duloxetine hydrochloride. J Taibah Uni Sci 2014; 8(4): 357-363.
15. Abdelhamid NS, Naguib IA, Anwar BH and Magdy MA: "A validated HPTLC method for the quantitative determination of duloxetine hydrochloride and 1-naphthol in bulk and pharmaceutical formulation. J Pla Chrom 2020; 33: 391-396.
16. ICH. (2005). Q2 (R1), harmonized tripartite guideline, validation of analytical procedures: text and methodology, In Proceedings of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use.
17. Kaur N, Mittal K, Nagar R, Nepali K and Thakkar A: A Sensitive spectrophotometric method for the determination of Pregabalin in pure drug and Pharmaceutical formulations through Benzoylation. Int Res J Pharm 2010; 1(1): 175-180.
18. Gee NS, Brown JP, Dissanayake VU, Offord J, Thurlow R and Woodruff GN: The novel anticonvulsant drug, gabapentin (Neurontin), binds to the alpha2delta subunit of a calcium channel. J Biol Chem 1996; 271(10): 5768-5776.

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