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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF PREGABALIN AND DULOXETINE IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT
A novel, efficient, and reproducible reverse-phase high-performance liquid chromatography (RP-HPLC) method has been developed and validated for the concurrent quantification of Pregabalin and Duloxetine in bulk materials and commercial pharmaceutical tablets. The chromatographic separation was carried out using an Agilent C-18 (100mmX 4.6mm, 2.5µm). The mobile phase was composed of methanol and acidified water (adjusted to pH 4.2 using 0.1% orthophosphoric acid and triethylamine) in a 45:55 (v/v) ratio, delivered at a constant flow rate of 1.0 mL/min. Detection was performed at a wavelength of 250 nm using a DAD detector. The method was validated according to ICH Q2(R1) guidelines, assessing key parameters such as specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantitation (LOQ). Pregabalin and Duloxetine showed retention times of approximately 3.177 minutes and 5.734 minutes, respectively. The method demonstrated excellent linearity across the concentration ranges of 15 µg/mL for Pregabalin and 4 µg/mL for Duloxetine, with correlation coefficients exceeding 0.999. Accuracy was confirmed through recovery studies, with results falling within the 98% to 102% range. This RP-HPLC method offers a straightforward and dependable approach for the simultaneous determination of Pregabalin and Duloxetine and is well-suited for use in routine quality control of combined dosage formulations.

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

INTRODUCTION
Pregabalin is a medication with anticonvulsant, analgesic, and anxiolytic properties, commonly prescribed for conditions such as epilepsy, neuropathic pain, fibromyalgia, opioid withdrawal symptoms, and generalized anxiety disorder (GAD). Although structurally similar to gamma-aminobutyric acid (GABA), it does not act directly on GABA receptors. Instead, its therapeutic effect is achieved through selective binding to the α2δ-1 subunit of voltage-gated calcium channels, reducing neurotransmitter release and neuronal excitability.

Duloxetine, on the other hand, is classified as a serotonin-norepinephrine reuptake inhibitor (SNRI), enhancing the levels of these neurotransmitters in the central nervous system to exert its antidepressant and analgesic effects. Pregabalin and Duloxetine are used in combination to manage neuropathic and chronic pain more effectively.

Furthermore, a robust, reliable, and efficient reverse-phase high-performance liquid chromatography (RP-HPLC) method has been successfully established and validated for the simultaneous estimation of Pregabalin and Duloxetine in both bulk drug substances and marketed tablet formulations.

Nevertheless, many methods are developed and validated for pregabalin and duloxetine as individual and as well as simultaneous. Technically, combined detection of Pregabalin and Duloxetine can exert some issue due to have not been sufficiently developed or validated simple, rapid and sensitive, stable and highly effective simple RP-HPLC method for determination of Pregabalin and Duloxetine.

HPLC Method Development
Methods for new products are developed when no official methods are available. Alternative methods for existing (non-pharmacopoia) products are to reduce cost and time for better precision and rugming. When the

proposed alternative method aims to change comparative laboratory data to change the existing process, including merit/demerits. The goal of HPLC-Methode is to try and separate, the main active drug, any response impurities, all available synthetic inter-status and any derogatory.[7]

3. MATERIAL AND METHODS

3.1 List of reagents & chemicals used

Table No 1: List of Reagents and Chemicals used.

Sr. No	Name of Chemicals	Manufacturer
1.	Methanol (HPLC Grade)	Merck Ltd., India
2.	Acetonitrile (HPLC Grade)	Merck Ltd., India
3.	Ortho-phosphoric Acid Buffer (HPLC Grade)	Merck Ltd., India
4.	Tri-ethyl Amine	Merck Ltd., India

3.2. Drug Profile

Table No. 2: Drug Profile of Pregabalin.

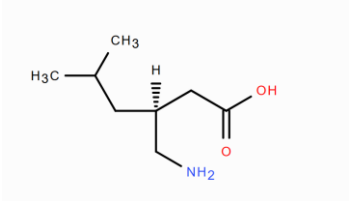
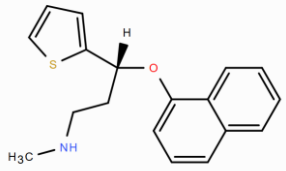
Particular	Pregabalin
Category	Anticonvulsant drug
Structure	
IUPAC Name	(3S)-3-(aminomethyl)-5-methylhexanoic acid
Molecular formula	C8H17NO2
Molecular weight	159.229 g·mol−1
CAS No.	148553-50-8
Melting point	228-229°C
Pka	12.6
Solubility	Water, methanol, ethanol, isopropanol
Protein Binding	1%
Half life	4.5–7 hours

Table No. 3: Drug Profile of Duloxetine.

Particular	Duloxetine
Category	Antidepressant drug
Structure	
IUPAC Name	(+)-(S)-N-Methyl-3-(naphthalen-1-yloxy)-3-(thiophen-2-yl)propan-1-amine
Molecular formula	C18H19NOS
Molecular weight	297.42 g·mol−1
CAS No.	116539-59-4
Solubility	Water, methanol, ethanol, isopropanol
Protein Binding	95%
Half life	12 hours

3.3 HPLC Method and Development: Chromatographic Condition and Instrument:

Table No. 4: Chromatographic conditions (HPLC) details used during method Development.

1.	HPLC	Agilent Tech. Gradient System with Auto injector
2.	Software	ChemStation
3.	Column	(Agilent) C18 column (4.6mm x 100mm)
4.	Particle size packing	2.5 □m
5.	Stationary phase	C-18 (Agilent)

6.	Mobile Phase	Methanol: Water (0.1% OPA) PH adjusted 4.2 With TEA 45: 55
7.	Detection Wavelength	250 nm
8.	Flow rate	1 ml/min
9.	Temperature	Ambient
10.	Sample size	20 □l
11.	pH	4.2
12.	Run Time	10 min
13.	Filter paper	0.45 □m

- **Preparation of std. Pregabalin solution: (Stock I)**

From the freshly prepared standard stock solution (750 µg/ml), 0.2 ml stock solution was pipette out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration of 15 µg/ml.

- **Preparation of std. Duloxetine solution: (Stock II)**

From the freshly prepared standard stock solution (200 µg/ml), 0.2 ml stock solution was pipette out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 4 µg/ml.

- **Preparation of std. Pregabalin and Duloxetine solution : (Stock III)**

From the freshly prepared standard stock solution (750 µg/ml:200 µg/ml), 0.1 ml stock solution was pipette out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 7.5 µg/ml of Pregabalin and 2 µg/ml was Duloxetine.

4. METHOD VALIDATION

The method of verification suggested in this study was validated for several parameters, including the test in the system suitability, specificity, accuracy, accuracy, linearity, detection range, volume limit, strength and analytical solution.

4.1 Study of System Suitability Parameter

System suitability is used to verify, is there sufficient for the determination of chromatographic system and reproduced to the eligibility analysis. The test was done by collecting data from five replica injections of the standard solution.

4.2 Linearity

The weight was weighed 75 mg pregabalin and 20 mg duloxetine weighing and transferred to 100 ml volumetric flask and deluxe to make volume. Sonicated for 10 minutes with topical rotation. The 0.1 mL of this solution was added to make the volume to dilute to 10 mL volumetric flask. The plot should be linear to pass through the original. Correlation coefficient should not be less than 0.999.

4.3 Accuracy (Recovery)

75 mg pregabalin and 20 mg duloxetine working standards were weighed and transferred to 100 ml volumetric flask and added to 10 mL to 10 mL with retarded, the volume of this solution was added to 100 mL volumetric flask and makable to make 0.1 mL of this solution. The second recovery should be within the range of 98-102%. Relative standard deviation should not exceed 2.0%.

4.4 Repeatability

75 mg pregabalin and 20 mg duloxetine were weighed and transferred to 10 mL volumetric flask and delicate was added to make volumes. Sonicated for 10 minutes with topical rotation. The above solution was filtered through the 0.45µm membrane filter. The accuracy of the system was determined with samples of RP-HPLC method. Two replicas of 75 mg pregabalin and 10 mg duloxetine containing sample solutions were injected and the summit areas were measured and %RSD was calculated. Was repeated five times.

4.5 Precision

The accuracy of an analytical method is the degree of agreement between individual testing results when the procedure is repeatedly applied to several samples of a similar sample. The accuracy of an analytical method is usually expressed as standard deviation or relative standard deviation. In addition, the results obtained were subjected to the ANOVA and the class within the day and the meaning of the day was determined and compared using F-Test. There were 75 mg pregabalin and 20 mg duloxetine working standards weighed and transferred to 10 mL volumetric flask & diluent was added to make up the volume. 0.1 ml of this solution diluted upto 10 ml with diluent.

4.6 Robustness

It was intentionally estimated by the protocol example flow rate, mobile phase and wavelength by ± 10%, ± 5%, and ± 2 nm respectively. Once the suitability of the system is installed according to the functioning, set the system suitability criteria once again and inject duplicate samples of sample solutions (samples prepared for method precision) in the functioning according to the strong study.

4.7 Detection Limit

Based on the S.D. of the response and the slope of calibration curve, the detection limit (DL) was calculated as,

$$DL = \frac{3.3\sigma}{S}$$

Where,

σ = the S.D. of the y-intercepts of regression lines.

S = the slope of the calibration curve.

The slope S may be estimated from the calibration curve and S.D. was used should be calculated from the y-intercepts of regression line in calibration curve.

4.7 Quantitation Limit

Based on the S.D. of the response and the slope of calibration curve, the quantitation limit (QL) was calculated as,

$$QL = \frac{10\sigma}{S}$$

Where,

σ = the S.D. of the y-intercepts of regression lines.

S = the slope of the calibration curve.

The slope S may be estimated from the calibration curve and S.D. was used should be calculated from the y-intercepts of regression line in calibration curve.

5. Analysis of marketing posterization

In order to determine the contents of pregabalin and duloxetine in marketing tablets (label claims 75 mg pregabalin and 20 mg duloxetine), 20 tablet powder was weighed in 2.7 grams and calculated in 135 grams of average weight of powder and was equal to 135 mg. It was sonic for 15 minutes to ensure complete extraction. The 0.5 ml surface was then thinner up to 10 ml with the mobile phase. The resulting solution was injected into HPLC and the drug peak area was mentioned.

The regression equation was generated using the peak areas of standard solutions. The amount of pregabalin and duloxetine in the sample was calculated using the regression equation and summit area of the sample. The amount of pregabalin and duloxetine was obtained from the regulation equation of the calibration curve as described in the analysis of the tablet.

6. RESULTS AND DISCUSSIONS

6.1 UV spectroscopy

The methanol produced UV absorption of 10 micrograms/mL solutions of pregabalin and duloxetine and absorbed in the range of 200–400 Nm. In methanol, λmax of pregabalin and duloxetine was found to be 216 nm and 284 nm respectively.

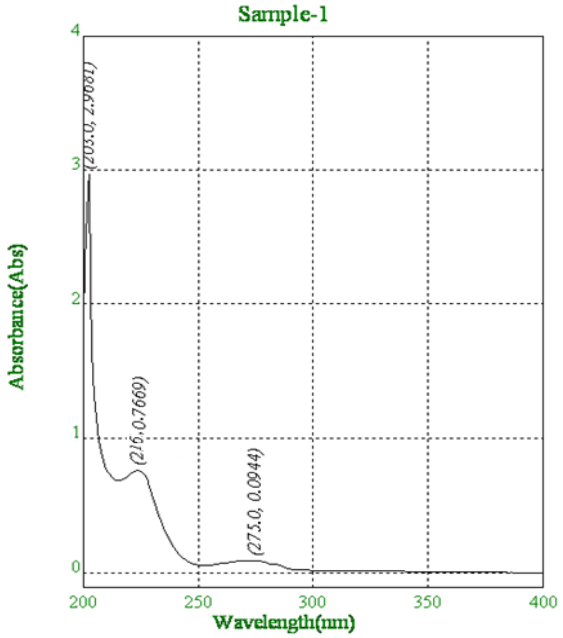


Fig. No. 1: UV Spectrum of Pregabalin.

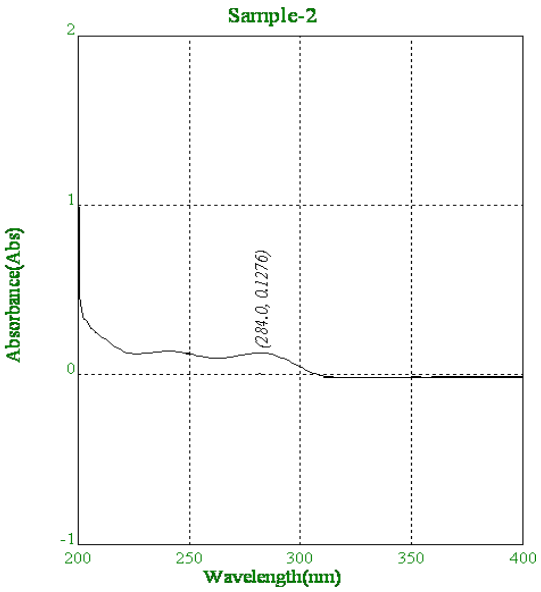


Fig. No. 2: UV Spectrum of Duloxetine.

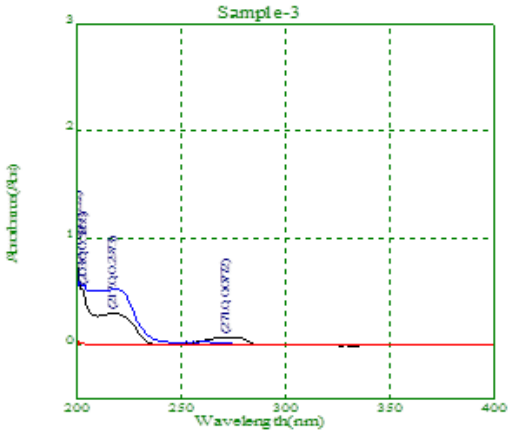


Fig. 3: ISO-absorptive point of Pregabalin and Duloxetine.

- The final chromatographic conditions selected were as follow:
- **Analytical column**: Agilent C18 Column (100mm x 4.6mm, 2.5µm partical size).
- **Injection volume** : 20µl
- **Flow rate** : 1 ml/min
- **Mobile phase** : Methanol: water (0.1%OPA) PH 4.2 with TEA
- **Detection** : 250nm
- Run Time : 10 min

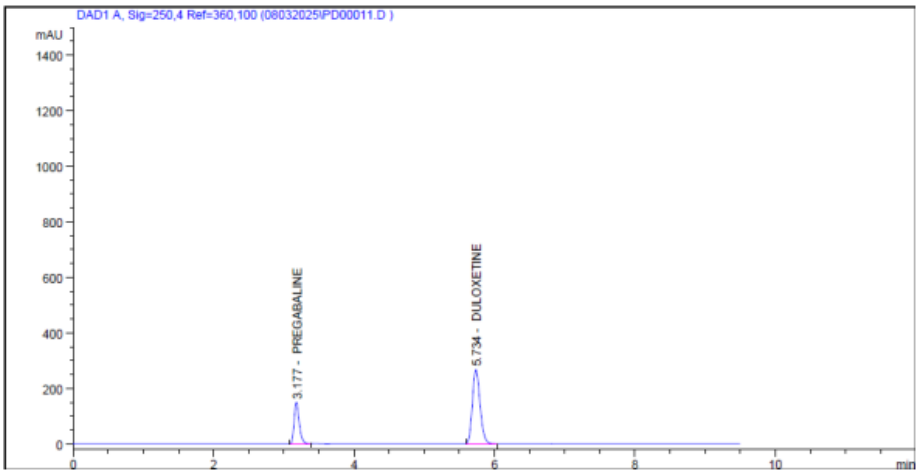


Fig. No. 4: Chromatogram of standard Combination of Pregabalin and Duloxetine.

Details of chromatogram of standard Combination containing Pregabalin and Duloxetine are follows

No.	RT [min]	Area[mV*s]	TP	TF	Resolution
1	3.177	767.48016	9633	0.71	0.0000
2	5.734	2030.62598	13852	0.80	15.74

Pregabalin and Duloxetine were found above 2000 in standard mixture of theoretical plates i.e. Pregabalin and Duloxetine at minimum RT 3.177 and 5.734 respectively for 9633 and 13852.

6.2. Analysis of law verification

1. Linearity

From the pregabalin standard stock solution, various tasks standard solutions (7.5-37.5µg/ml) were prepared in the mobile phase, similarly different functions from duloxetine standard stock solution were prepared in the standard solution (2–10µg/ml) in the mobile phase. Chromatograms were recorded. The area was recorded for each concentration (Table No. 5,6). The calibration is shown in declining figs. Number 5,6).

Table No 5. Linearity of Pregabalin.

Concentration µg/ml	Area Pregabalin
7.5	767.03
15	1411.91
22.5	2107.28
30	2734.75
37.5	3448.00

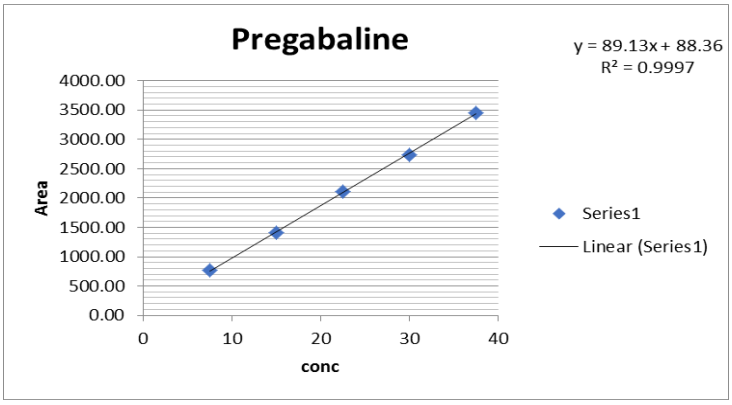


Fig. No. 5: Calibration curve of Pregabalin for HPLC method.

Table No. 6: Regression equation data for Pregabalin.

Regression Equation Data Y=mx+c	
Slope(m)	89.13
Intercept(c)	88.36
Correlation Coefficient	0.999

Table No. 7: Linearity of Duloxetine.

Concentration µg/ml	Area Duloxetine
2	2030.62
4	3679.27
6	5469.59
8	7039.34
10	8700.89

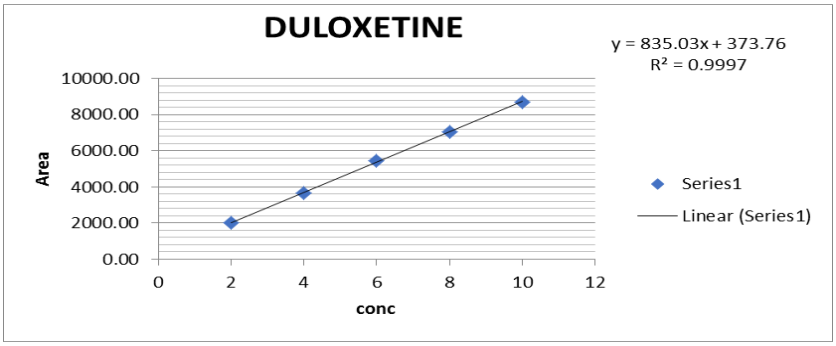


Fig. No. 6: Calibration graph of Duloxetine for HPLC method.

Table 8: Regression equation data for Duloxetine.

Regression Equation Data Y=mx+c	
Slope(m)	835.0
Intercept(c)	373.7
Correlation Coefficient	0.999

The linearity of pregabalin and duloxetine was seen in the range of 7.5–37.5 µg/ml and 2–10µg/ml. The detection wavelength used was 250nm. The plot must pass through the original; Correlation coefficient should not be less than 0.999. This was concluded. (Table. No. 8).

2. Accuracy

Recovery studies were done to validate the accuracy of the developed method. To analyze the tablet solution, a certain concentration of standard medicine (80%, 100%and 120%) was added and then analyzed its recovery (Table No. 9). Statistical verification of recover studies shown in (Table No. 10).

Table 9: Result of Recovery data for Pregabalin and Duloxetine.

METHOD	Drug	Level (%)	Amt. taken (µg/ml	Amt. Added (µg/ml	Area Mean* ± S.D.	Amt. recovered Mean *±S.D.	%Recovery Mean *± S.D.
RP-HPLC Method	PGB	80%	7.5	6	13.32±0.033	5.82±0.033	98.23±0.32
		100%	7.5	7.5	14.99±0.004	7.49±0.004	99.93±0.05
		120%	7.5	9	16.55±0.003	9.05±0.003	100.51±0.03
	DXT	80%	2	1.6	3.59±0.004	1.59±0.004	99.32±0.25
		100%	2	2	3.99±0.004	1.99±0.004	99.29±0.22
		120%	2	2.4	4.43±0.001	2.43±0.001	101.20±0.04

*Mean of each 2 reading for RP-HPLC method

Table 10: Statistical Validation of Recovery Studies Pregabalin and Duloxetine.

METHOD	Level of Recovery (%)	Drug	Mean % Recovery	Standard Deviation*	% RSD
RP-HPLC Method	80%	PGB	98.23	0.32	0.32
		DXT	99.32	0.25	0.25
	100%	PGB	99.93	0.05	0.05
		DXT	99.29	0.22	0.22

	120%	**PGB**	100.51	0.03	0.03
		DXT	101.20	0.04	0.04

*Denotes average of three determinations for RP-HPLC

The accuracy of RP-HPLC method is detected by recovering concentrations of different levels (80%, 100%and 120%). % Recovery was found within 98–102 % (table number 9,10).

3. System Suitability Parameter: (recurrence)

The pregabalin and duloxetine system was studied to detect the resolution and copy of the proposed chromatographic system for assessment of suitability parameters. The result shown below (Table No. 11).

Table No. 11: Repeatability studies on RP-HPLC for Pregabalin and Duloxetine.

Method	Concentration of Pregabalin and Duloxetine (mg/ml)	Peak area	Amount found (mg)	% Amount found
RP-HPLC Method for PBG	22.5	2123.3	22.84	101.47
	22.5	2122.99	22.82	101.48
		Mean	22.83	101.46
		SD	0.22	0.22
		%RSD	0.01	0.01
RP-HPLC Method for DXT	6	5474.95	6.12	101.68
	6	5460.7	6.08	101.68
		Mean	6.10	101.68
		SD	10.08	10.08
		%RSD	0.18	0.18

Recovery studies on RP-HPLC for Pregabalin and Duloxetine were found to be 101.46 and 101.68%, the% RSD was less than 2%, which indicates a high percentage amount found between 98%to 102%that indicates an analytical method that concluded. (Table No. 11).

4. Precision

The method was established by analyzing various replication standards of pregabalin and duloxetine. All solutions were analyzed three times to record any intra-day and inter-day variation in the result. The results for intraday are shown in (Table No. 1) respectively.

Table No. 12: Result of Intra day and Inter day Precision studies on RP-HPLC for Pregabalin and Duloxetine.

METHOD	Drug	Conc^{n} (µg/ml)	Intraday Precision		Interday Precision	
			Mean± SD	%Amt Found	Mean± SD	%Amt Found
Rp- HPLC METHOD	**DXT**	2	2054.04±5.38	100.62	2065.30±5.33	101.29
		6	5467.48±10.68	101.67	5479.87±0.52	101.92
		10	8722.32±24.04	99.98	8681.56±9.19	99.50
	PBG	75	763.16±3.54	100.95	762.21±0.11	100.82
		22.5	2125.54±2.83	101.58	2125.32±5.66	101.57
		37.5	3463.06±4.19	100.97	3430.95±3.14	100.01

***Mean of every 3 reading for RP-HPLC**

Intrade and Inter Day on RP-HPLC for pregabalin and duloxetine indicate the high precision% amount between 98% to 102%, which indicates the analytical method concluded.

5. Robustness

The strengthening parameters of a method have the ability to remain unaffected by small intentional changes. To evaluate the strength of the proposed method, small but intentional changes were made in customized method parameters. The effect of changes in the mobile phase structure and flow rate, the retention was studied at the time wavelength and the tail factor of the drug peak.

The mobile phase structure was converted to (± 1 mL/min) ratio and differed from the flow rate (± 1 mL/min), and wavelength changes of customized chromatographic position (± 1 mL/min). The consequences of the study of strength are shown in (Table No.13,14). Broadness parameters were also found satisfactory; Hence the analytical method will end.

Table No. 13: Result of Robustness Study of Duloxetine.

Parameters	Conc.(µg/ml)	Amount of detected (mean ±SD)	%RSD
Chromatogram of flow change 0.9ml	4	4479.04±27.00	0.60
Chromatogram of flow change 1.1 ml	4	3230.67±10.25	0.32
Chromatogram of comp change 44 ml Meoh+56ml OPA	4	3814.8±11.35	0.30

Water			
Chromatogram of comp change 46 ml Methanol+54 ml OPA Water	4	3847.39±26.76	0.70
Chromatogram of comp change wavelength change 249 nm	4	4235.7±2.41	0.06
Chromatogram of comp change wavelength change 251 nm	4	3464.52±27.29	0.81

Robustness Study of Duloxetine

Changes to flow rates (± 1 mL/ min), mobile phase structure (± 1 mL/ min), and wavelength (± 1 mL/ min). %RSD was calculated for the peak area, which should be less than 2%. The result shown in the resulting method was concluded. (Table No. 13).

Table No. 14: Result of Robustness Study of Pregabalin.

Parameters	Conc. (µg/ml)	Amount of detected (mean ±SD)	% RSD
Chromatogram of flow change 0.9ml	15	1712.83±20.4	1.19
Chromatogram of flow change 1.1 ml	15	1210.32±6.92	0.57
Chromatogram of comp change 44 ml Meoh+56ml OPA Water	15	1472.8±16.64	1.13
Chromatogram of comp change 46 ml Methanol+54 ml OPA Water	15	1436.06±3.45	0.24
Chromatogram of comp change wavelength change 249 nm	15	1509.0±7.84	0.52
Chromatogram of comp change wavelength change 251 nm	15	1424.44±2.02	0.14

Robustness Study of Pregabalin

Changes to flow rates (± 1 mL/ min), mobile phase structure (± 1 mL/ min), and wavelength (± 1 mL/ min). %RSD was calculated for the peak sector, which should be less than 2%. Results shown in the analytical method (Table No. 14).

6. Limit Detection

The LOD is the lowest limit that can be detected. Based on the S.D. deviation of the response and the slope The limit of detection (LOD) may be expressed as:

LOD = 3.3 (SD)/S

where, SD = Standard deviation of Y intercept

S = Slope

Limit of detection = 0.1639 (µg/mL) of Pregabalin

Limit of detection = 0.180647 (µg/mL)of Duloxetine

The LOD of Pregabalin and Duloxetine was found to be 0.1639 (µg/mL) and 0.18064 (µg/mL), analytical method that concluded**.**

7. Limit Quantification

The LOQ is the lowest concentration that can be quantitatively measured. Based on the S.D. deviation of the response and the slope,

The quantitation limit (LOQ) may be expressed as:

LOQ = 10 (SD)/ S

Where, SD = Standard deviation Y intercept

S = Slope

Limit of Quantitation = 0.49695 (µg/mL)

Limit of Quantitation = 0.18064 (µg/mL)

The LOQ of Pregabalin and Duloxetine was found to be 0.4969 µg/mL) and 0.18064(µg/mL), analytical method that concluded.

8.3 Analysis of tablet formulation

Procedure

Weigh 20 pregabalin and duloxetine combination pills and transfer the average weight, weight of accuracy and 135 mg pregabalin and duloxetine and transfer the sample equal to duloxetine to 100 mL volumetric flask. To dissolve it, add about 100 ml methanol of diluent and sonicate and make volume to the mark with diluent. Mix well and filter through 0.45 µm filter. Thin to the scar with a pipette and retardation of 0.5 mL of the above stock solution in 10 mL volumetric flask. (37.5µg/ml and 10µg/mL). The test is shown in the simple chromatogram (Figure: 6) of pregabalin and duloxetine, the amount of pregabalin and dulexetine of the tablet was calculated by extraditing the value of the area from the calibration curve. The analysis process was repeated five times with tablet construction. For calculating %RSD for Tablet assay %RSD, the result (Table No. 15) was shown in.

Brand Name: PG More Dulo (Osvel pharma)

Total weight of 20-tab Powder wt. = 2.7 gms

Avg Powder Weight = 135 gms./Tab

Eq.Wt for 75 mg = 75 x 135/ 75 = 135 mg

1) **Take 135 mgs in 10 ml Methanol. = 750 µgm/ml PGB and 200 µgm/ml DXT–II**

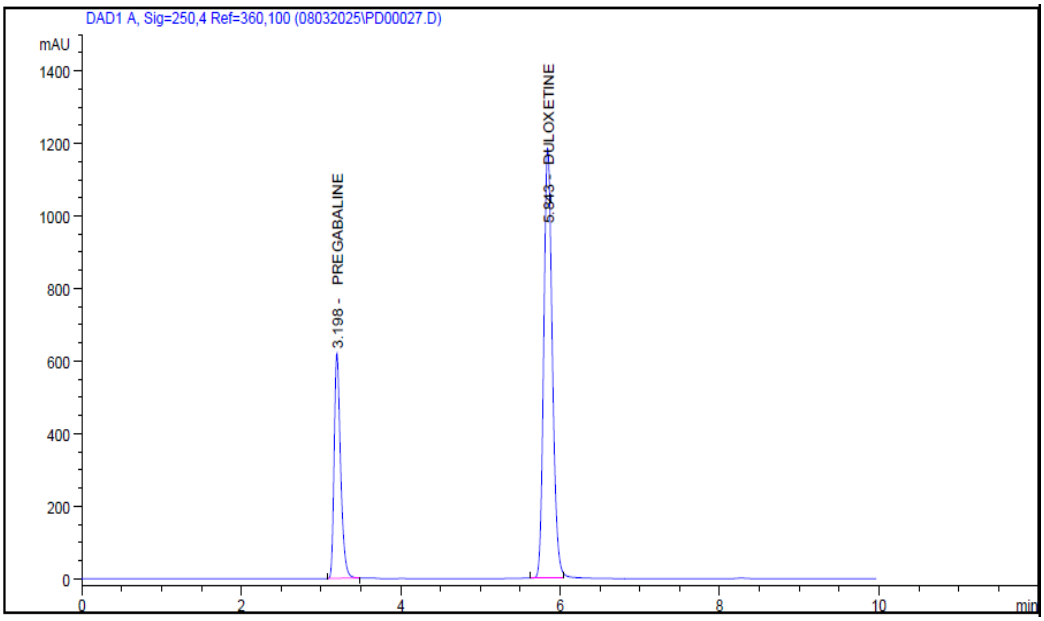


Fig No.6: Chromatogram for Marketed Formulation (mcg)

Table No. 15. Details of Chromatogram of Marketed Formulation (37.5+10 mcg)

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	3.198	3477.43384	8161	0.69	0.0000
2	5.843	8833.85449	14725	0.81	15.80

Table 16. Analysis of marketed formulation.

Assay	Drug	Conc	Area	% Lable Claim	SD	%RSD
Rp-HPLC Method	PGB	37.50	3477.43	101.40	0.158	0.417
	DXT	10	8833.85	101.32	0.014	0.141
	PGB	37.50	3457.51	100.80	0.42	0.417
	DXT	10	8816.94	101.12	0.143	0.14

Analysis of marketed formulation were also %Label Claim was found to be 98-101% Satisfactory are concluded. (Table No. 16).

9. Ruggedness

The degree of reproducibility of test result obtains by the analysis of same sample under variety of Condition. Such as different analyst, laboratory Different instrument.

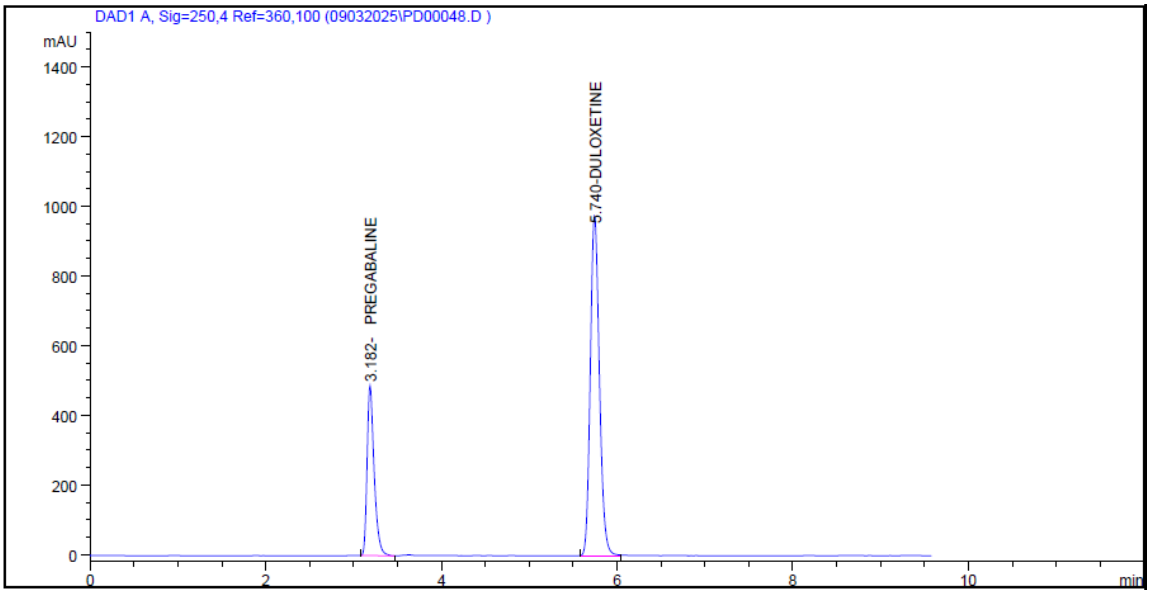


Fig. No.7: Chromatogram for Analyst-1 (30+8 mcg).

Table No. 17: Analysis of Analyst-1 (30+8 mcg).

R. T	AREA	TH. PLATES	SYMM
3.182	2726.85632	8325	0.65
5.740	7022.45630	15263	0.81

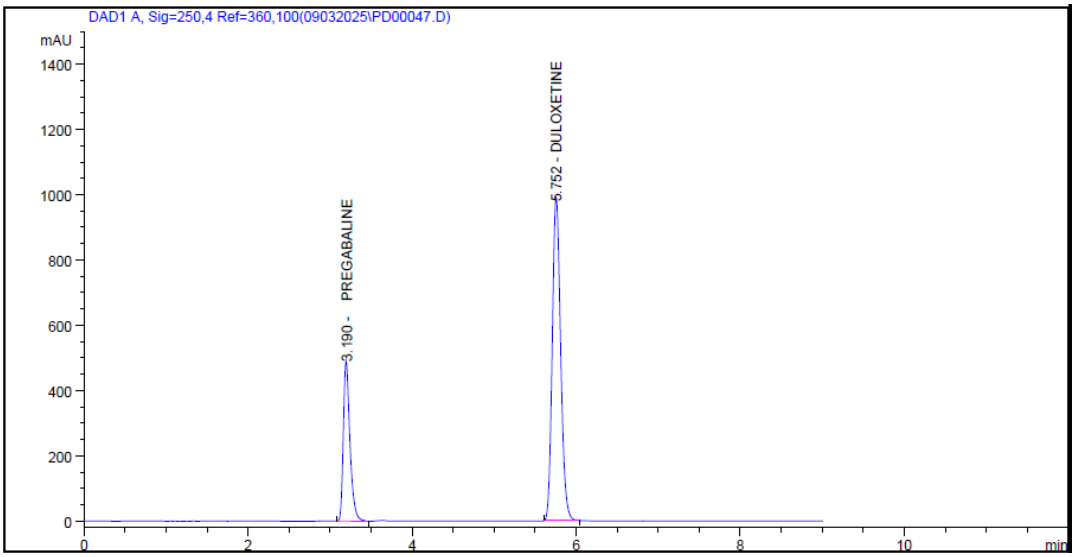


Fig No.8: Chromatogram for Analyst-II (30+8 mcg).

Table No. 18: Analysis of Analyst-II (30+8 mcg).

R. T	AREA	TH. PLATES	SYMM
3.190	2729.45200	8362	0.66
5.752	7024.78631	15723	0.82

Specificity and Selectivity

Analysis should not have any interference from external components and should be well resolved. In the presence of the specificity component, there is a quantitative analyst detection process that may be expected to be present in the sample matrix, while the selection is a qualitative analyzer detection process in the presence of components that may be expected to be present in the sample matrix.

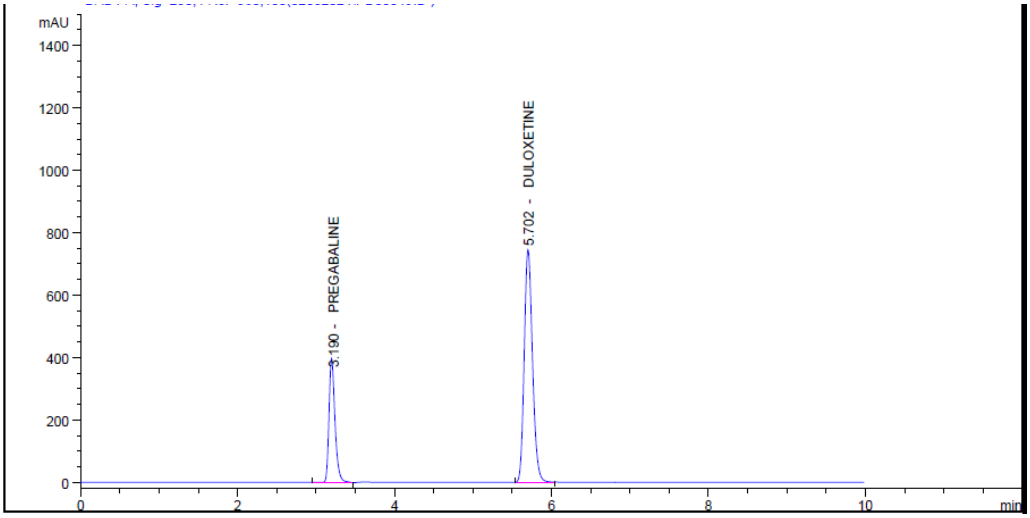


Fig. No 9: Chromatogram of Specificity and Selectivity (22.5+6 mcg).

Table No 19: Details of Chromatogram of Specificity and Selectivity.

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	3.190	2120.57780	9563	0.72	-
2	5.702	5470.12304	15321	0.80	15.64

CONCLUSION

Official methods for determining pregabalin and duloxetine in pharmaceutical dose form are scheduled in the United States Pharmacopoeia (USP). For the determination of pregabalin and duloxetine as powder, HPLC method, particle size 5 mm, length 4.6x 250 mm, methanol and acetonitrile are used as a mobile phase using a reversed phase column with methanol and acetonitrile. Pregabalin and Duloxetine standard and PDA address at 250 Nm and at 1ml/min flow rate. An RP-HPLC method was developed and verified for related and related substances.

The validity of liquid chromatographic assay was established through a study of linearity, system precision, intermediate precision, accuracy, strength. The linearity was installed with a series of work solutions by dilute the stock solution with weakening for the final concentration of 80%, 100% and 120%. Each concentration was injected into liquid chromatography and the price of the peak area was taken to the calibration curve. The calibration curve was plotted using concentration against the peak area. The correlation co-efficient value was found to be 0.999, indicating that the concentration of pregabalin and duloxetine had good linearis. In accurate studies, % RSD 0.38 and 0.46 for pregabalin and Duloxetine was found. The result indicates that the method is valid for accuracy. In intermediate accurate studies, % RSD 0.13 and 0.12 for pregabalin and duloxetine were found. There is no significant difference by other analysts by different time intervals on different days. Therefore, the intermediate precision of the method may be acceptable. In accuracy or recovery studies, overall % of recovery and % RSD for P

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