Section A-Research Paper
ISSN 2063-5346

A SIMPLE ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF IMPURITY AND FORCED DEGRATION STUDIES OF LEVOMILNACIPRAN HYDROCHLORIDE BY RP-HPLC

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Abstract:
Levomilnacipran is used as an antidepressant and Levo enantiomer of Milnacipran which is used for the treatment of fibromyalgia. The RP-HPLC method was developed by using Symmetry C-18, 150*4.6mm, 3.5µm column with mobile phase A of Buffer which is made up of orthophosphoric acid and mobile phase B was made up of water, and acetonitrile in the ratio of 90: 10% v/v. The diluent taken was water and methanol in the ratio of 50:50% v/v. Flow rate was 1.0µL/min, UV detection at 210nm with PDA detector andthe injection volume was 10 µL and the run time was 50mins. This method was validated with respect to Phthalimido impurity for specificity and found no interference of any other substances. Linearity studies were done for phthalimido impurity from 0.47ppm to 3.15ppm and found correlation coefficient as 0.998. Precision was performed and found %RSD as 0.7. Accuracy was also performed from 50% to 150% and recovery found above 92%. Robustness and ruggedness were found in limits. The linearity of Levomilnacipran was found from 9.6µg/ml to 22.4µg/ml. Accuracy for Phthalimido impurity was performed from 50% to 150% and recovery was found to be in limits. Degradation studies were performed to know the amount of drug degraded during acid, base, peroxide, humidity, thermal and photolytic stresses. It was found that the drug was degraded more in peroxide stress.

Key Words: Levomilnacipran, Phthalimido impurity, %RSD, Acid stress, Base stress, Photolytic stress, Peroxide stress etc...

1. INTRODUCTION:

Levomilnacipran used as an antidepressant which was approved in the United States in 2013 to treatment major depressive disorder (MDD). It is the Levo enantiomer of Milnacipran which is used for the treatment of fibromyalgia. Levomilnacipran has similar effects and pharmacology of Milnacipran, acting as a serotonin–norepinephrine reuptake inhibitor (SNRI). Its IUPAC name is (1S,2R)-2-(amino methyl)-N, N-diethyl-1-phenylcyclopropane-1- carboxamide hydrochloride. Levomilnacipran binds with high affinity to the human serotonin (5-HT) and norepinephrine (NE) transporters (Ki = 11 and 91 nm., respectively) and potently inhibits 5-HT and NE reuptake (IC50 = 16-19 and 11 nm., respectively). Levomilnacipran lacks significant affinity for any other receptors, ion channels or transporters tested in vitro, including serotonergic (5HT1-7), α- and βadrenergic, muscarinic, or histaminergic receptors and Ca2+, Na+, K+ or Cl- channels. Levomilnacipran did not inhibit monoamine oxidase (MAO). The concentration of levomilnacipran at steady state is proportional to dose when administered from 25 to 300 mg once daily. Following an oral administration, the mean apparent total clearance of

levomilnacipran is 21-29 L/h. Steady-state concentrations of levomilnacipran are predictable from single-dose data. The apparent terminal elimination half-life of levomilnacipran is approximately 12 hours.

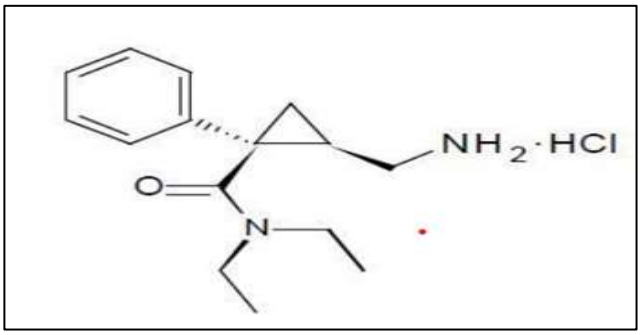
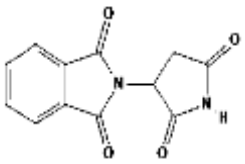


Figure No: 1. Showing the structure of Levomilnacipran.

Levomilnacipran consists of an inhouse Thalimide impurity which is process related.

Tabulation no: 1. showing the impurity Profile of Levomilnacipran

Chemical Name	Structure	In- house	Type ofimpurity
(1S,2R)-2-((1,3-dioxoisoindolin-2- yl)methyl)-N,N diethyl-1-phenylcyclopropane-1-carboxamide		Phthalimido impurity	Processrelated

Maximum Daily dose for levomilnacipran Capsules –120mg. Fetzima is the brand name under which marketed formulation is available with label claim of 20, 40, 80 and 120 mg. Literature suggests that many methods were existed for estimation of Milnacipran in bulk and dosage forms by HPLC but methods for estimation, impurity and stability studies of Levomilnacipran were very less. BRC Sekhar Reddy et al (2014), proposed a stability indicating RP-HPLC method for estimation of levomilnacipran but not estimated impurity.[1] Surve, Bhushan Vijay et al (2016), proposed a Assay method development and validation of Levomilnacipran active pharmaceutical ingredients by Reverse phase HPLC but does not gives an idea about impurity profiling and stability studies.[2] M Hakiful Haque et al (2020), proposed a zero order and first order spectrophotometric method for determination of levomilnacipran, no study has done on stability and impurity profiling.[3] Badithala Siva SaiKiran and Sundararajan Raja (2018)[4], Priti j. Mehta* and deepak m. Khatri et.al (2010)[5], Palleppagari Sridhar, et.al (2015) proposed HPLC methods for estimation of milnacipran in bulk and dosage forms but levomilnacipran was not estimated[6]. Kanchan Nautiyal and K. Ramakrishna (2014) proposed impurity profiling on milnacipran [7] and Naresh Tondepu et al., proposed a stability indicating method for milnacipran but does not estimate levomilnacipran.[8] This method was developed to estimate levomilnacipran, impurity and carrying out stability studies which was advanced and simple.

2. MATERIALS AND METHODS:

2.1. Materials:

Chemicals:

Water, acetonitrile, orthophosphoric acid, methanol and levomilnacipran.

Instruments:
Analytical balance Satoris – CP255d, Sonicator- PCI Analytics – model 35 LIR, HPLC Agilent open lab control, Centrifuge R-8M Remi.

2.2. Methods:
Many trails had performed to optimise chromatic conditions but the following conditions shows best optimization.

Tabulation No: 2. showing Optimized Chromatographic conditions

Optimized Chromatographic conditions		
1	Column	Symmetry C-18, (150*4.6mm, 3.5µm)
2	Flow rate	1.0ml/min
3	Detection	210nm
4	Injection volume	10µl
5	Column temperature	40□C
6	Run time	50minutes
7	Sampler temperature	25□C

Mobile phase A: 0.5% orthophosphoric Acid
Mobile phase B: Water and Acetonitrile 10:90 %v/v
Diluent: Water and Methanol 50:50 %v/v

Tabulation No: 3. showing Gradient Programme

Time (min)	**0.0**	**2.0**	**40.0**	**45.0**	**45.1**	**50.0**
% of MP – A	90	90	0	0	90	90
% of MP – B	10	10	100	100	10	10

Based on the data, there was no interference was observed from blank, placebo and degradants at retention time of Levomilnacipran and its related compounds. Peak symmetry and sharpness for Levomilnacipran and its related compounds was found satisfactory. Based on the above method development trails, below mentioned final method is developed for related compound of Levomilnacipran Capsules which shall be used for the routine analysis.

Preparation of Mobile Phase A:
Transferred 5.0mL of Ortho phosphoric acid into a 1000mL volumetric flask, diluted tovolume with water and mix.

Preparation of Mobile Phase B: Prepared a degassed mixture of Acetonitrile: Water in the ratio of 90 : 10%v/v.

Diluent: Mix methanol and water in the ratio of 50 :50%v/v.

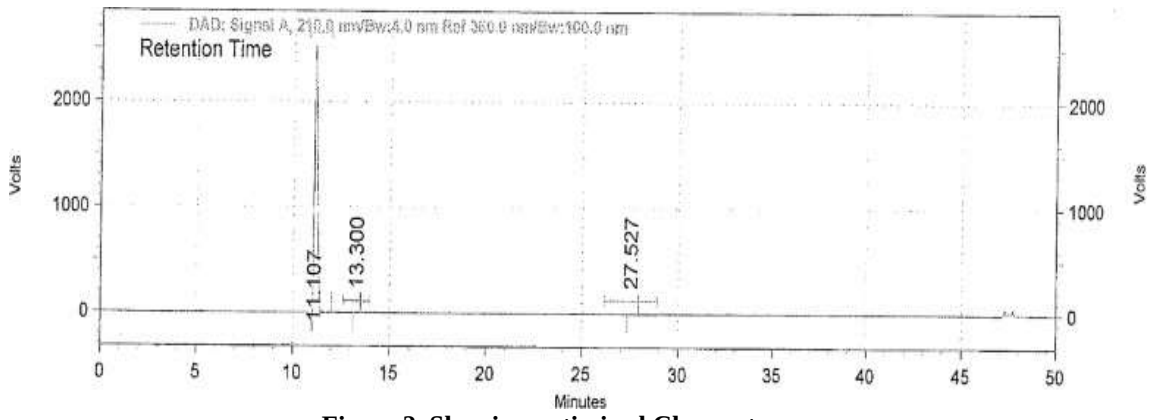


Fig no:2. Showing optimized Chromatogram

Tabulation No: 4. showing Retention times and Area of Levomilnacipran and Phthalimido impurity:

Sno.	Name	Retention Time	Area
1	Levomilnacipran	11.107	3713804024
2	Phthalimido impurity	27.52	14693789
3	Unknown impurity	13.3	75051472

3. RESULTS AND DISCUSSIONS:

3.1. Analytical Method Validation:

3.1.1. Specificity:

The HPLC chromatograms recorded for the drug-matrix (mixture of the drug and excipients) showedalmost no interfering peaks with in retention time ranges. The figures show that the selected drug was well separated. Thus, the HPLC method proposed in this study was specific.

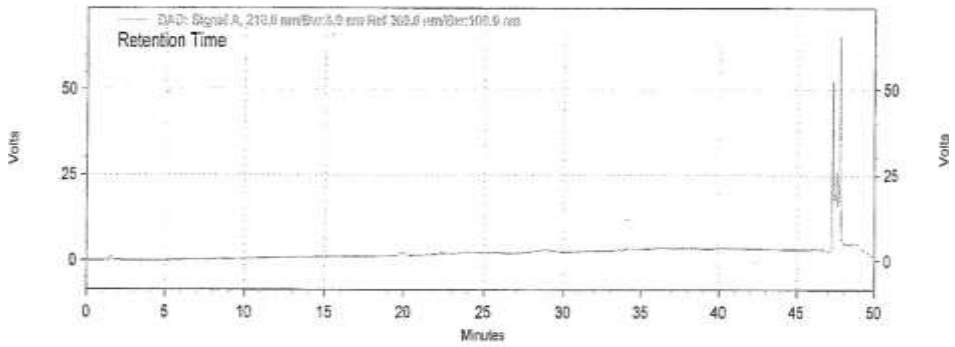


Fig no:3. Showing blank Chromatogram

Showing Chromatogram for placebo

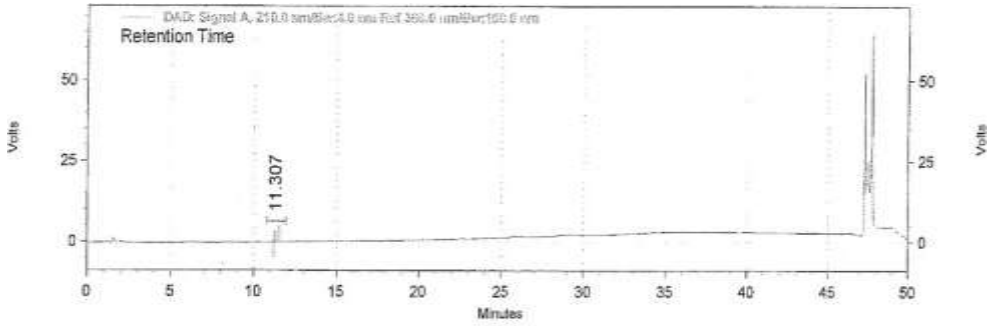


Fig no: 4. Showing Chromatogram for levomilnacipran standard

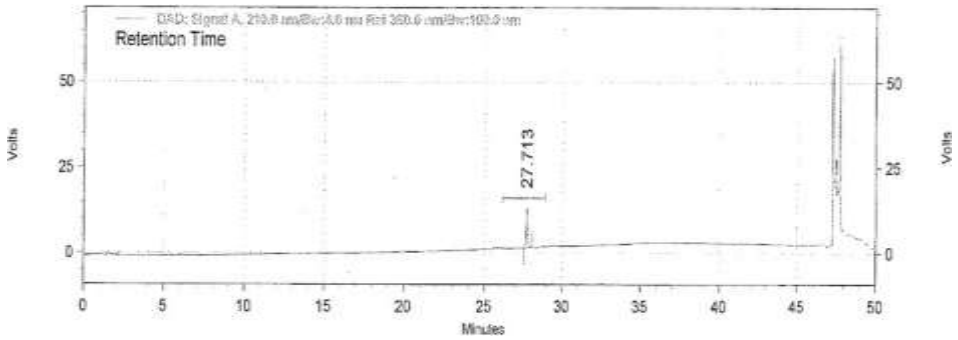


Fig no: 5. Showing Chromatogram for Phthalimido impurity

3.1.2. Precision:

System precision: The standard solutions were prepared and injected as six replicate injections into the HPLC, the percentage RSD for the area of the main peak was 2.0
Method precision: Method precision was performed on six sample preparations spiked with phthalimido impurity, the variation was found to be with in the acceptance criteria. 2.0

Tabulation No: 5. showing Precision for Levomilnacipran and Impurity percentage

S.No	Spike level	Area	% Impurity
1	100% - 1	13342012	0.175
2	100% -2	13476996	0.176
3	100% -3	13427517	0.176
4	100% - 4	13274098	0.174
5	100% -5	13252203	0.173
6	100% -6	13336472	0.175
AVG		13351549.67	0.174833333
STDEV		86827.00299	0.001169045
%RSD		0.65	0.67

3.1.3. Linearity:

The linearity was performed using the standard solution from the concentration levels ranging from 50% to 150% over six levels and linearity for phthalimido impurity in a spiked solution was done and found the regression in very acceptable limits.

Tabulation No: 6. showing Linearity of Levomilnacipran

S.No.	Concentration (ppm)	Area
1	0.47	3290321
2	0.95	6558946
3	1.58	10823905
4	1.97	13581025
5	2.36	16289335
6	3.15	21464401
Correlation Coefficient		0.9999

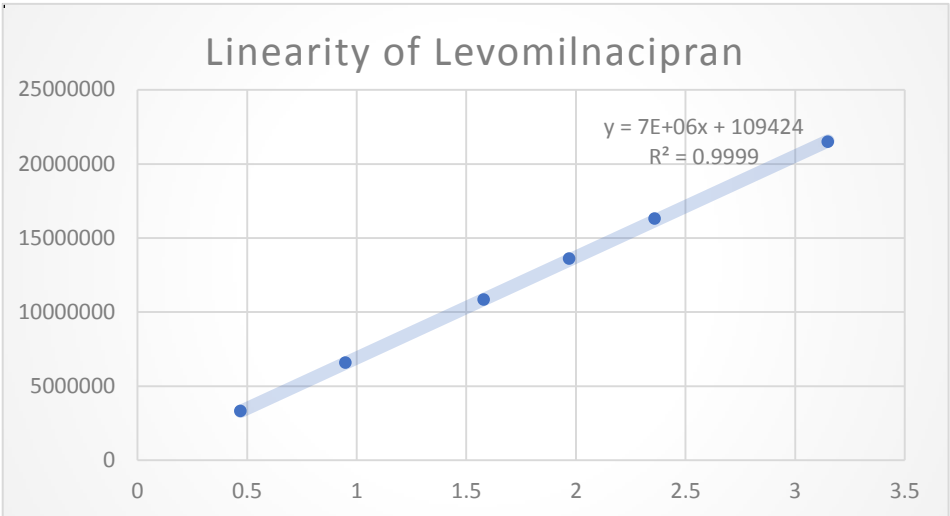


Figure No: 6. Graph showing Linearity of Levomilnacipran

Tabulation No: 7. showing Linearity of Phthalimido impurity:

S.No.	Concentration (ppm)	Area
1	0.47	1970375
2	0.95	3852811
3	1.58	6311600
4	1.97	7994567
5	2.36	9689359
6	3.15	12712701
Correlation Coefficient		0.9996

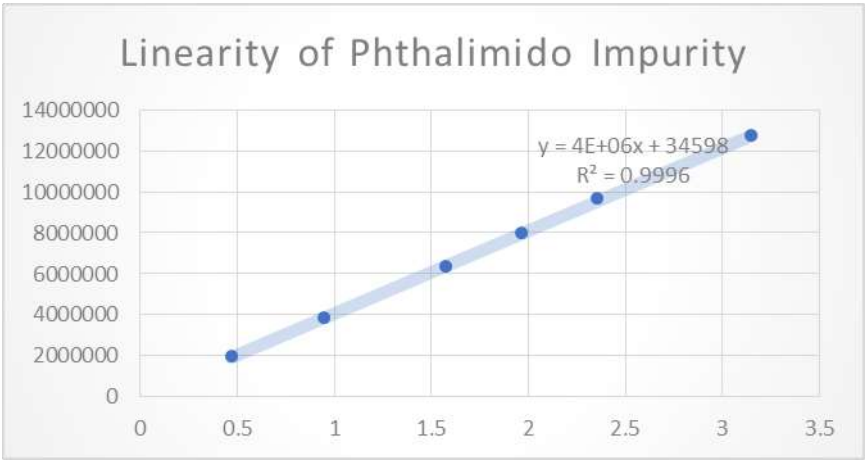


Figure No: 7. Graph showing Linearity of Phthalimido impurity

3.1.4. Accuracy:

Accuracy was performed for Levomilnacipran from 50% to 150% concentrations and found the recoveries 98%, 98.6% and 97.9% respectively. Accuracy studies for the Phthalimido impurity was also performed by spiking 50%, 100% and 150% concentrations and found the recoveries as 95.75%, 92.46% and 92.51% respectively.

Tabulation No: 8. showing Accuracy for Phthalimido impurity

S.N0	Accuracy level	Amount added	Amount found	% Recovery	Mean Recovery	%RSD
1	50%	0.095	0.089	93.68	95.75	1.9
2		0.095	0.092	96.84		

3		0.095	0.092	96.84		
1	100%	0.190	0.175	92.11	92.46	0.3
2		0.190	0.176	92.63		
3		0.190	0.176	92.63		
1	150%	0.285	0.263	92.28	92.51	0.2
2		0.285	0.264	92.63		
3		0.285	0.263	92.51		

4. FORCED DEGRADATION STUDIES:

The samples were subjected to forced degradation in the following conditions
Degradation conditions:

Tabulation No: 9. Showing degradation studies:

Stress Condition	Condition
Acid stress	30 ml of 2N HCl at 80^0 C for 2hrs
Base stress	30ml of 2N NaOH at 80^0 C for 2hrs
Peroxide stress	45ml of 33% peroxide on bench top for 2hrs
Humidity stress	95% for 2 days
Thermal stress	Kept in oven at 80^0 C for 24hrs
UV light	For 2hrs

4.1. Acid Stress:

Acid stress was performed by taking 57.33mg of levomilnacipran and add 20ml of diluent, sonicate for 15minutes add 30ml of 2NHCl reflux for 2hours at 80°C. Neutralized the solution with 2N NaOH and make up the volume upto 100ml using diluent. Sample as such was also taken and assayed.

Tabulation No. 10. showing Acid stress details

Sample	Wt. (mg)	Diluent (ml)	Sonication (min)	2NHCl (ml)	Reflux at 80°C	2N NaOH (ml)	Made Upto with diluent (ml)
As such	57.09	70	30	NA	NA	NA	100
Acid stress	57.33	20	15	30	2hrs	30	1000

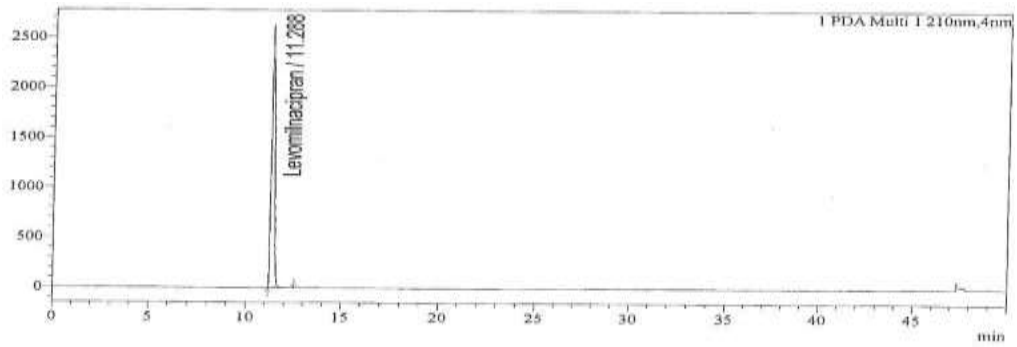


Fig No: 8. showing Chromatogram of levomilnacipran as such

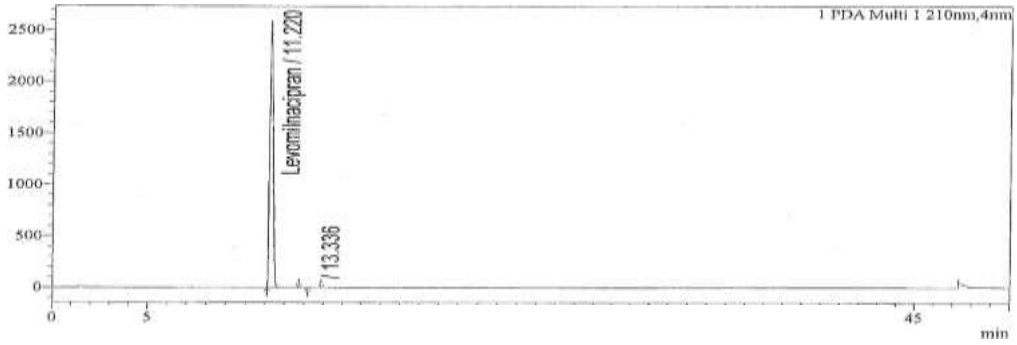


Fig No:9. showing Chromatogram of levomilnacipran Acid stress

4.2. Base Stress:

Tabulation No. 11. showing Base stress details:

Condition	Wt. (mg)	Diluent (ml)	sonication (min)	2N NaOH	Reflux at 80°C	2N HCl	Made up to with diluent (ml)
Base stress	57.12	20	15	30	2 hrs	30	1000

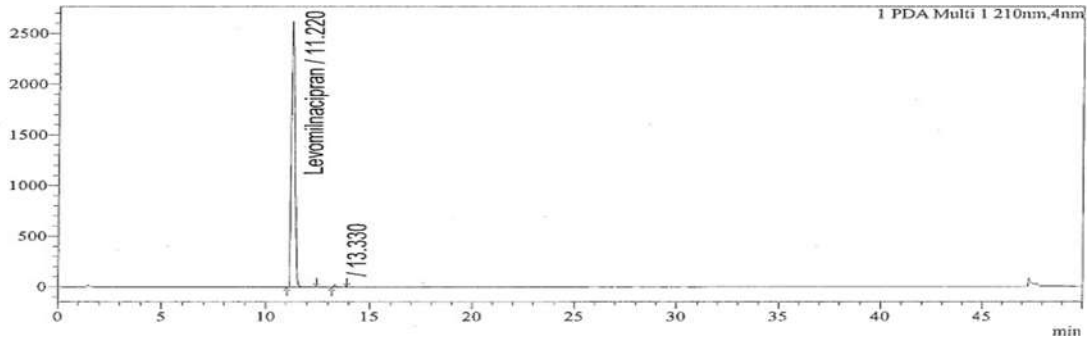


Fig No: 10. showing Chromatogram of levomilnacipran Base stress

4.3. Peroxide stress:

Tabulation No. 12. showing Peroxide stress details:

Condition	Wt. (mg)	Diluent (ml)	sonication (min)	33% H_2O_2	Bench top (hrs)	Made up to with diluent (ml)
Peroxide stress	57.41	20	15	40	2	1000

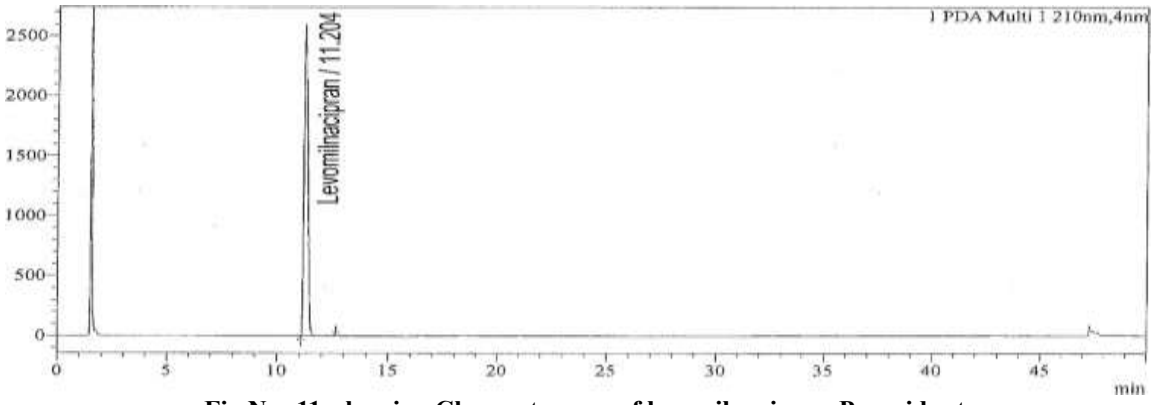


Fig No: 11. showing Chromatogram of levomilnacipran Peroxide stress

4.4. Thermal Stress:

Tabulation No. 13. showing Thermal stress details:

Condition	Wt. (mg)	Temp	Time	Diluent	Sonication (min)	Made up to with diluent (ml)
Heat stress	57.02	100°C	24 hrs	180	30	1000

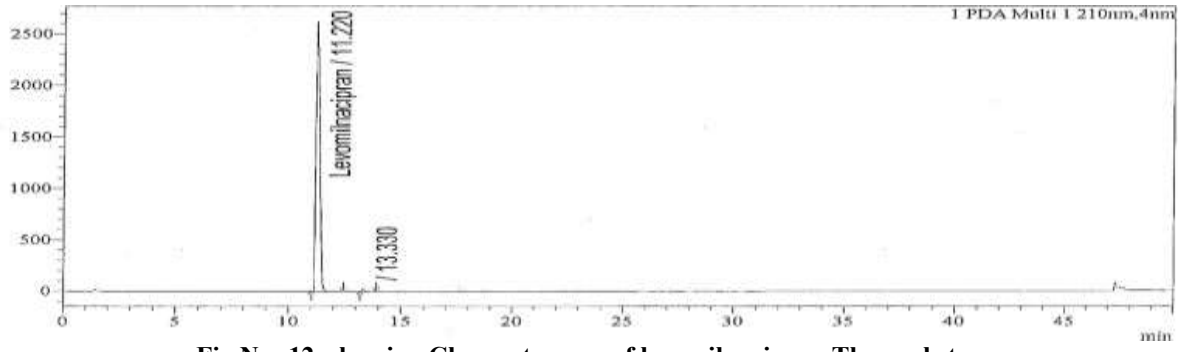


Fig No: 12. showing Chromatogram of levomilnacipran Thermal stress

4.5. Humidity Stress:

Tabulation No. 14. showing Humidity stress details:

Condition	Wt. (mg)	Humidity	Diluent	Time	Sonication (min)	Made up to with diluent(ml)
Humidity Stress	57.19	95%	180	24hrs	30	1000

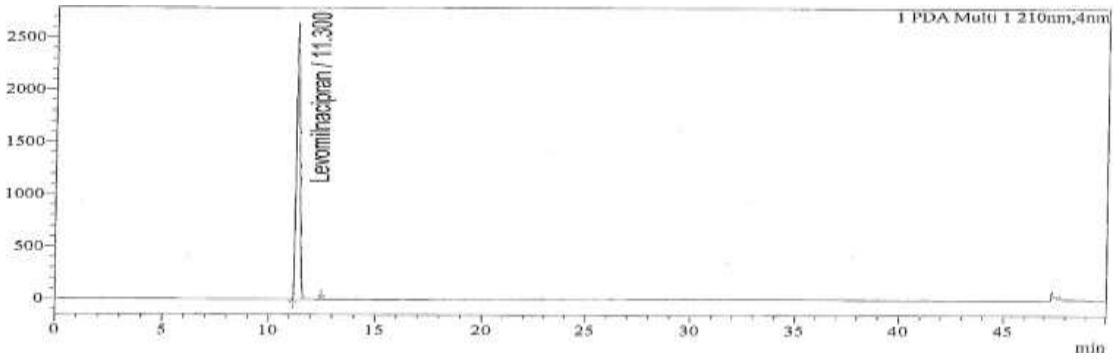


Fig No: 13. showing Chromatogram of levomilnacipran Humidity stress

4.6. UV Light stress:

Tabulation No. 15. showing UV stress details:

Condition	Wt. (mg)	UV light exposure (hrs)	Diluent	Sonication (min)	Made up to with diluent(ml)
UV light	57.55	2	180	30	1000

Note: The above stress sample centrifuged at 5000 RPM for 10minutes and the supernatant solution injected into HPLC. For Mass Balance, from the above solution solutions further diluted to 4.0mL to 10mL for assay concentration.

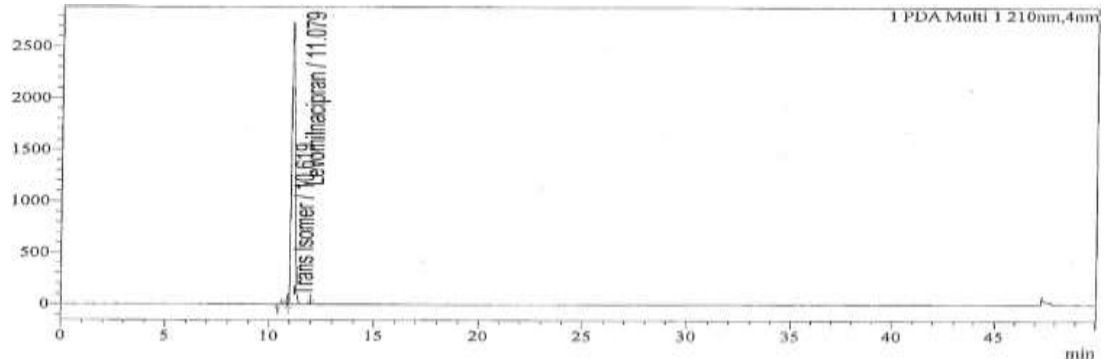


Fig No: 14. showing Chromatogram of levomilnacipran UV light exposure stress

4.7. Results of stress conditions:

Tabulation No. 16. showing Degradation in different stress conditions

Name	%Assay	RS	Mass balance
As such	100.4	-	NA
Acid stress	99.4	0.095	99.09
Base stress	99.1	0.457	99.16
Peroxide stress	99.2	-	98.74
Humidity stress	100.1	-	99.62

Photolytic stress	98.5	0.097	98.79
Thermal stress	100.4	-	99.96

5. DISCUSSION:

The RP-HPLC method was developed by using Symmetry C-18, 150*4.6mm, 3.5µm column with mobile phase A of Buffer which is made up of orthophosphoric acid and mobile phase B was made up of water, and acetonitrile in the ratio of 90: 10% v/v. The diluent taken was water and methanol in the ratio of 50:50% v/v. Flow rate was 1.0µL/min, UV detection at 210nm with PDA detector andthe injection volume was 10 µL and the run time was 50mins.The developed method was validated by using various parameters according to ICH guidelines. All the validation parameters were found to be good with acceptance criteria. The system suitability parameters also reveals that the values within the specified limit for the proposed method. The theoretical plates for Levomilnacipran hydrochloride were found to be not less than 5000 and the tailing factor was fond to be not more than 2.0. The retention time of the Levomilnacipran and impurity peak were found to be. 11.10 minutes and 27.52 minutes respectively. The precision of the system and the method were checked and found to be within the limits.

A new RP-HPLC method has been developed and validated for the estimation of related substances of in levomilnacipran dosage form. It is shown that the above method was found to be accurate, linear, precise, robust and reproducible. This method was validated with respect to Phthalimido impurity for specificity and found no interference of any other substances. Linearity studies were done for phthalimido impurity from 0.47ppm to 3.15ppm and found correlation coefficient as 0.998. Precision was performed and found %RSD as 0.7. Accuracy was also performed from 50% to 150% and recovery found above 92%. Robustness and ruggedness were found in limits. The linearity of Levomilnacipran was found from 9.6µg/ml to 22.4µg/ml. Accuracy for Phthalimido impurity was performed from 50% to 150% and recovery was found to be in limits. Degradation studies were performed to know the amount of drug degraded during acid, base, peroxide, humidity, thermal and photolytic stresses. It was found that the drug was degraded more in peroxide stress.

6. CONCLUSION:

The developed method was very simple, accurate and precise for estimation of Levomilnacipran and its impurity. This method also serves best for its degradation studies. Hence this method can be practiced for daily analysis in academic institutions as well as in industry.

Declarations

Ethics approval and consent to participate:
Not applicable.

Consent for publication:
All the authors approved the manuscript for publication.

Availability of data and material:
All required data is available.

Competing interests:
All authors declare no competing interests.

Funding:
Not applicable.

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