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Development and Validation of RP-HPLC Method for the Estimation of Levo Milnacipran

Badithala Siva Sai Kiran[1], Sundararajan Raja[2].
GITAM Institute of Pharmacy[1, 2], GITAM (Deemed To Be University), Visakhapatnam-530045, India
Email: sivasaikiran143@gmail.com[1]

Abstract- A rapid and precise reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed for the estimation of Levo (L) Milnacipran. Chromatography was carried out on a phenomenex C18 (4.6 x 250mm, 5µm) column using a mixture of water: methanol (65:35% v/v) as the mobile phase at a flow rate of 1.0mL/min, the detection was carried out at 262 nm. The retention time of the L Milnacipran was 2.97 min respectively. The method produces a linear response in the concentration range of 15-90 mg/mL of L Milnacipran. The method precision for the determination of assay was below 2.0% RSD. The method is useful in the routine analysis of bulk drug of L Milnacipran. The validation of the developed method was performed according to ICH guidelines.

Keywords: L Milnacipran, RP-HPLC, ICH, Validation.

1. INTRODUCTION

L Milnacipran is the first in a new class of serotonin-norepinephrine reuptake inhibitor. It is an antidepressant and also used in the clinical treatment of fibromyalgia[1-3]. L Milnacipran inhibits norepinephrine uptake with approximately three folds higher potency in vitro than serotonin without directly affecting the uptake of dopamine or other neurotransmitters [4-6]. Chemically it is [2-(aminomethyl)-N, N-diethyl-1-phenylcyclopropane carboxamide] hydrochloride as shown in Figure 1 with empirical formula $C_{15}H_{22}N_2O$ and molecular weight 246.34g/mol. There are various methods are available for the estimation of L Milnacipran hydrochloride in pharmaceutical dosage forms using HPLC [7-11], LC/MS [12], GC-MS [13] U-HPLC [14-15] and Spectrophotometry [16]. However, there were no reported methods for estimation of L Milnacipran API. So sincere effort was made to develop a simple chromatography method for estimation of L Milnacipran and validated as per ICH [17] guidelines to prove the developed method was accurate and Precise

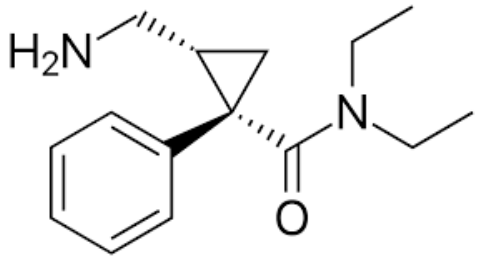

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Fig No 1: Structure of L Milnacipran

2. MATERIALS AND METHOD:

The L Milnacipran was kindly supplied as a gift sample by varun herbals (Hyderabad) Limited. All agents and chemicals used were of Analytical grade.

Table No 1: List of Materials

S.No	CHEMICALS	MANUFACTURER
1	Levo milnacipran (API)	Varun Herbals, Hyderabad
2	Methanol (HPLC grade)	Merck , Mumbai, India
3	Water (HPLC grade)	Merck , Mumbai, India
4	Distilled water	Milli Q system (In house)

Table No 2: List of Equipment

S.No	INSTRUMENTS	MODEL NO	MANUFACTURER
1	UFLC	LC 20 AD PDA detector	Shimadzu., japan
2	Ultra Sonicator	2200 MH Soltech	SPINCOTECH Pvt., ltd
3	Digital balance	NO-HT 220	VIBRA Shinko Denshi co.ltd
4	Ultra Filtration		Millipore pvt.ltd.

2.1. Preparation of Standard Stock Solution:

10 mg of L Milnacipran pure drug was weighed and transferred into 10mL volumetric flask and add 10mL of mobile phase (1000µg/mL concentration). From this stock solution various aliquots are prepared and injected.

2.2. Optimization of Method

The proper selection of methods depends upon the nature of the sample (ionic or ionisable or neutral molecule) its molecular weight and stability. The drug selected is polar, ionic and hence Reversed phase chromatography was carried out on a phenomanixC18 (4.6 x 250mm, 5µm) column using a mixture of Methanol: Water (35: 65 v/v) as the mobile phase at a flow rate of 1mL/min, the detection was carried out at 262nm. The retention time of the L Milnacipran was 2.97 min respectively.

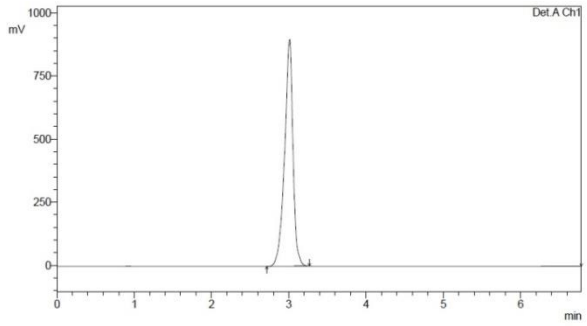

Fig No 2: Optimized Chromatogram of L. Milnacipran

3. RESULTS AND DISCUSSION

3.1. Specificity:

3.1.1. Specificity by Direct comparison method

There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analyte.

Table No. 3: Specificity Data

S.No	Peak Name	Observation
1	Blank	Nil
2	Placebo	Nil
3	Standard	Rt : 2.97min

3.2. *System Suitability:*

System suitability test was an integral part of method development and has been used to ensure adequate performance of the chromatographic system.

Table no: 4 Results of System Suitability

Parameter	Result	Acceptance Limit
Retention time (Rt)	2.97min	--
Resolution factor	NA	--
Number of theoretical plates (N)	2462	More than 2000
Tailing factor (T)	1.16	Less than 2
Number of injections: 6 replicates		

3.3. Precision:

Results for intraday and interday precision

Table no: 5 Precision results of L Milnacipran

S.No.	Intraday Precision Peak Area	Interday Precision Peak Area
1	831605	841324
2	819564	851262
3	823545	823486
4	830546	830442
5	826984	846620
6	801350	820545
Mean	822265.7	835613.2
Std Dev	10201.72	11543.46
%RSD	1.24	1.38

3.4. Linearity and Range

Table No: 6 Results of calibration curve at for L Milnacipran

S. No	Concentration (µg/mL)	Peak Area
1	15	210656
2	30	421452
3	45	610665
4	60	824563
5	75	1051546
6	90	1239871

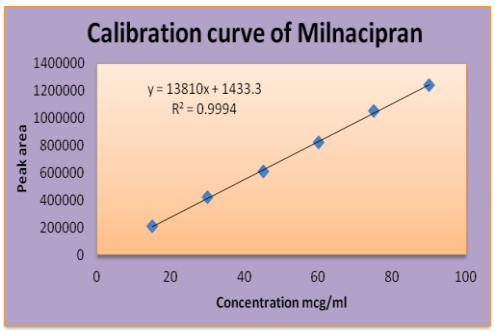

Fig no 3: Calibration curve of L Milnacipran

3.5. Accuracy:

Accuracy of the method was determined by Recovery studies.

Table no: 7 Determination of Accuracy results for L Milnacipran

Spiked Concentration (µg/mL)	Peak area	Amount added (µg/mL)	Amount Found (µg/mL)	Recovery	% Mean Recovery
30	413540	29.96	29.85	99.65	100.83
	421452		30.42	101.55	
	420345		30.34	101.29	
60	819564	59.92	59.16	98.74	99.34
	823545		59.45	99.22	
	830546		59.96	100.06	
90	1223158	89.88	88.30	98.24	99.42
	1239871		89.51	99.59	
	1250546		90.28	100.44	

3.6. LOD & LOQ:

Table No.8: Results for LOD & LOQ

S.NO	Parameter	Slope	Standard Deviation	Value
1	Limit of Detection	13810	10201.72	2.44

3.7. Robustness:

Table no: 9 Results of Robustness Studies
Change of Flow Rate (± 10%)

S.No	Flow Rate	0.9mL/min	1mL/min	1.1mL/min
1		851262	823545	830546
2		823486	831546	826984
3		830442	810684	801350
4	Mean	835063.33	821925	819626.7
5	Std dev	11800.96	8593.56	13005.11
6	% RSD	1.41	1.04	1.586712

Table no: 10 Results of Robustness Studies
Change in Temperature (± 5°C)

S.No	Temperature	30 °C	35 °C	40 °C
1		851262	820486	830442
2		823486	835442	846620
3		830442	840620	820545
4	Mean	835063.33	832182.7	832535.7
5	Std dev	11800.96	8536.66	10747.53
6	% RSD	1.41	1.02	1.29

3.8. *Assay (% Purity)*:

The assay of L Milnacipran was found to be 100.9854 **%**

4. CONCLUSION:

The proposed study describes a novel RP-HPLC method for the estimation of L Milnacipran. The method gives good resolution for the compound with a short analysis time (<3 min). The method was validated and found to be simple, rapid, selective, accurate and precise when compared to the reported methods. Percentage of recovery shows that the method is free from interference. The method is also cost effective with respect to solvent consumption. Therefore, the proposed method can be used for routine analysis of L Milnacipran.

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