Research Article

NEW METHOD DEVELOPMENT AND VALIDATION OF AN RP-HPLC METHOD FOR THE DETERMINATION OF LEVOMILNACIPRAN IN BULK AND PHARMACEUTICAL DOSAGE FORM.

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ABSTRACT

To establish sensitive and accurate methods for developing and validating analytical methods for estimation of levomilnacipran in bulk and pharmaceutical dosage forms. A mixture of 10 mM Dipotassium hydrogen phosphate buffer pH 6.5 and Methanol in the ratio of 50:50 (v/v%) was used as the mobile phase. A working standard solution of a concentration of 20 µg/ml was used. An XBridge™ C18 column 5µ (250 mm x 4.6 mm) was used for the analysis at a flow rate of 1 ml/min, injection volume of 20 µl, run time of 10 mins, and detection wavelength of 210 nm. The %RSD values of precision studies were found to be below the accepted limit of 2%. The method was found to be linear with a correlation coefficient (r^2) of 0.998. The method was also found to be accurate and robust with suitable values. The LOD and LOQ of the method were found to be 1.42 µg/ml and 4.75 µg/ml respectively.

Keywords: levomilnacipran, RP-HPLC, validation, LOD, LOQ.

INTRODUCTION

Major depressive disorder (MDD) is a common psychiatric illness that affects approximately 30% of individuals residing in the United States within their lifetime.[1] The consequences of untreated depression can include significant difficulties in social and occupational functioning as well as risk of suicide and other self-destructive behaviors.[2] Levomilnacipran hydrochloride sustained release (Fetzima, Forest Laboratories) is a SNRI approved for treatment of MDD in July 2013.[3] It is also currently under investigation for use in functional recovery of patients after acute ischemic stroke in Europe.[4] Levomilnacipran is a capsule containing extended-release beads. Levomilnacipran is the more active enantiomer of milnacipran.[5] Milnacipran was approved by the Food and Drug Administration (FDA) in 2009 for the treatment of fibromyalgia.[6] Cumulative data from studies have found modest benefits in depression with the use of milnacipran; it has not been approved for depression management in the United States, but has been approved in other countries such as France for this indication.[7] Levomilnacipran is categorized as a SNRI. Similar to other agents in the SNRI class, levomilnacipran displays selective affinity for serotonin and norepinephrine transporters. The drug is the more potent of the enantiomers found in racemic milnacipran.[5] Its affinity for both norepinephrine and serotonin is higher than that of all the other SNRIs except for duloxetine. [8,9,10]

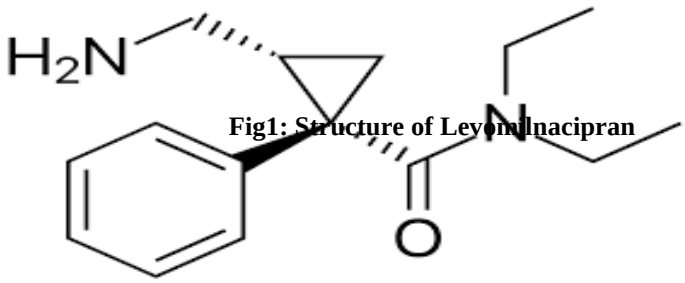

Fig1: Structure of Levomilnacipran

MATERIALS AND METHOD

Collection of reagents and solvents:

Levomilnacipran was obtained as a gift sample from Manus Aktteva Biopharma LLP, Ahmedabad, Gujarat, India. Levomilnacipran extended-release capsules dosage form each containing 40mg of levomilnacipran. Pharmaceutical formulation levomilnacipran extended-release capsules (label claim contains 40 mg) was used in HPLC analysis.

Table 1: chemicals and instrumentation

chemicals	Brand name
levomilnacipran	Manus Aktteva biopharma
HPLC grade Methanol	Merck
Dipotassium hydrogen phosphate	Merck
Water	Milford

Analytical method validation:

VALIDATION PARAMETERS

❖ **Accuracy**

The procedure for the preparation of the solutions for Accuracy determination at 80%, 100%, and 120% levels was prepared in methanol. For 80% Accuracy for Levomilnacipran: 32mg of the pure drug was added to 40mg of formulation. for 100% Accuracy for Levomilnacipran:40mg of the pure drug is added to 40mg of formulation. For 120% Accuracy Levomilnacipran:48mg of the pure drug is added to 40 mg of formulation. %Recovery and %RSD is measured. for each determination fresh sample is prepared and accuracy was calculated.

Acceptance criteria: should be within 98 -102%

Precision

The precision of the assay was determined in terms of intraday and Interday variation in the peak area for a set of drug solutions of 20µg/ml, assayed six times on the same day and different 2 days. The intraday and Interday variation in the peak ratio of the drug solution was calculated in terms of coefficient of variation (CV) and obtained by multiplying the ratio of the standard deviation to the mean by100 is shown in the graph.

(CV=SD/MEAN × 100.

Acceptance criteria: %RSD should be less than 2.

Linearity and range:

By using the working standard, aliquots of 05µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 25µg/ml, 30µg/ml, were prepared with Methanol and Buffer (50;50). Six dilutions of each of the above-mentioned concentrations were prepared separately and from these six dilutions, 20µl of each concentration was injected into the HPLC system. Then their chromatogram was recorded. Peak areas were recorded for all the peaks and a standard calibration curve of peak area against concentration was plotted.

Limit of detection and limit of quantification

LOD and LOQ were calculated according to ICH recommendations where the approach is based on the signal–to–noise ratio. Chromatogram signals obtained with known low concentrations of analytes were compared with the signals of the blank samples. A signal–to–noise ratio of 3:1 and 10:1 was considered for calculating LOD and LOQ respectively.

Robustness

As defined by the ICH, the robustness of analytical procedures describes its capability to remain unaffected by small and deliberate variation in the chromatographic conditions and found to be unaffected by small variation ±0.1ml/min in flow rate of mobile phase, and wavelength ±1nm result is shown.

System Suitability

The resolution, number of theoretical plates, Capacity Factor, S/N (6 Sigma) and peak asymmetry were calculated for the standard solutions.

Chromatographic conditions

XBridge™ C18 column 5µ (250 mm x 4.6 mm) was used for the analysis. The flow rate was set at 1 ml/min with a run time of 10 mins. The injection volume was 20 µl. The detector was set at a wavelength of 210 nm.

RESULTS AND DISCUSSION

SOLVENTS	LEVOMILNACIPRAN
Methanol	Soluble
Ethanol	Freely soluble
Water	Sparingly soluble
acetonitrile	Freely soluble

Assay

Twenty capsules, each containing 40mg of Levomilnacipran were weighed and finely powdered. A quantity of powder equivalent to 50 mg of Levomilnacipran was weighed and transferred to 50 ml volumetric flask containing 30 ml Methanol. The mixture was sonicated for 20 min. The volume was made up to 50 ml with Methanol. The contents were filtered through 0.45µ membrane filter. Further dilutions were made to get a concentration of 20µg/ml. Twenty microliters of the test and standard solutions were injected separately and chromatograms were recorded up to 10 min. The proposed method was found to be specific and no interference from capsule excipients was observed.

$$\text{Assay} = \frac{\text{Peak area of sample}}{\text{Peak area of standard}} \times 100$$

Injection 20 µg/ml	Peak Area
1	18581354
2	18611534
3	18436742
4	18527402
5	18582924

6	18396593
Average	18522758.17
Standard Deviation	87490.05
RSD (%)	0.47

Table 10: Assay of Levomilnacipran

Injection 20 µg/ml	Area
1	18444068
2	18331619
3	18310214
4	18172296
5	18544531
6	18669939
Average	18412111.17
Standard Deviation	178552.39
RSD (%)	0.97

Table 3: Intraday precision of Levomilnacipran (morning)

Injection 20 µg/ml	Area
1	18435750
2	18698702
3	18310214
4	18272296
5	18544531
6	18669939
Average	18488572.00
Standard Deviation	179753.76
RSD (%)	0.97

Table 4: Intraday precision of Levomilnacipran (afternoon)

Injection 20 µg/ml	Area
1	18611787
2	18425770
3	18882973
4	18809578

5	18568722
6	18693988
Average	18665469.67
Standard Deviation	166484.88
RSD (%)	0.89

Table 5: Intraday precision of Levomilnacipran (Day 1)

Injection 20 µg/ml	**Area**
1	18091781
2	18285213
3	18293946
4	18485488
5	18475454
6	18275069
Average	18317825.17
Standard Deviation	146639.35
RSD (%)	0.80

Table 6: Intraday precision of Levomilnacipran (Day 2)

Accuracy

Level of Percentage recovery	**80%**	**100%**	**120%**
Amount present (mg/tablet)	40	40	40
Amount of standard drug added(mg)	32	40	48
	14543037	18451642	22035985
Area response	14697391	18364987	22180046
	14703256	18292377	22075345
Mean	14647894.67	18369668.67	22097125.33
Standard Deviation	90856.74	79735.65	74459.25
RSD	0.62	0.43	0.34
Total amount recovery (mg)	71.77	80.00	88.23
% Recovery	99.69	100.01	100.26

Table 7: Accuracy for Levomilnacipran

Linearity:

By using the working standard, aliquots of 05µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 25µg/ml, 30µg/ml, were prepared with Methanol and Buffer (50;50). Six dilutions of each of the above-mentioned concentrations were prepared separately and from these six dilutions, 20µl of each concentration was injected into the HPLC system. Then their chromatogram was recorded. Peak areas were recorded for all the peaks and a standard calibration curve of peak area against concentration was plotted.

Concentration (µg/ml^{-1})	Area
5	4349649
10	8285566
15	12834720
20	18367390
25	22329732
30	26871928

Table 2: Linearity data for Levomilnacipran

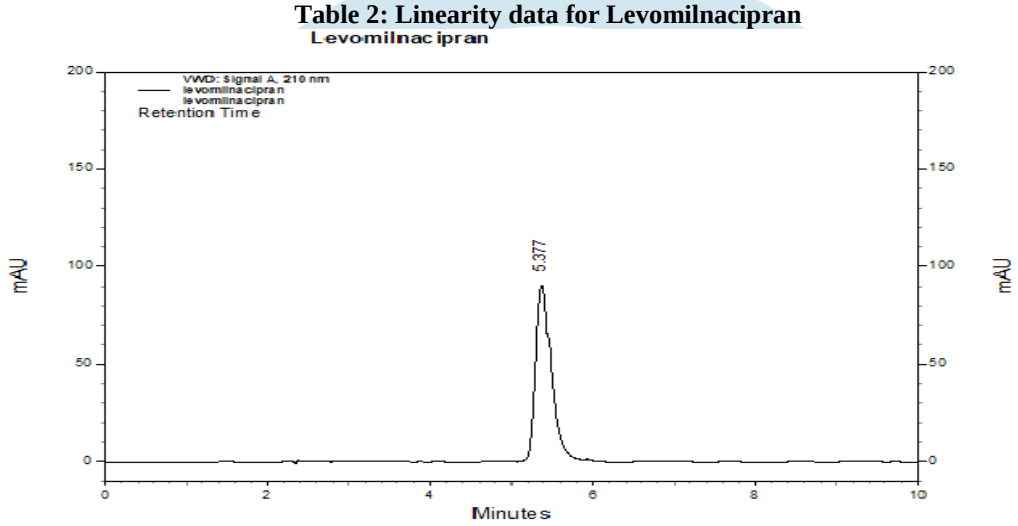

Figure 2: Optimized chromatogram standard

Linearity Graph

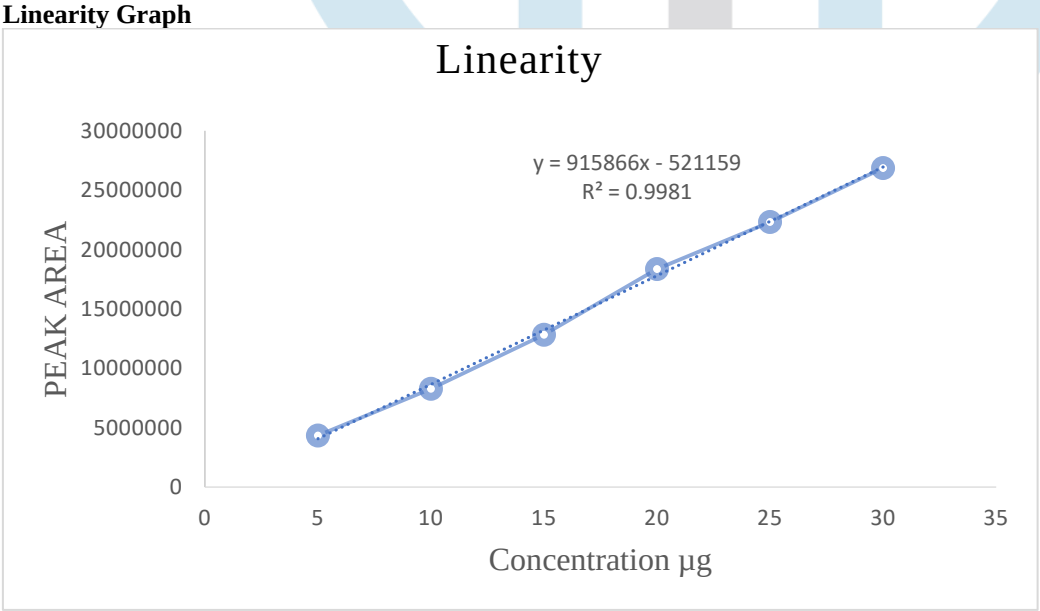

Figure 3: Linearity Graph of Levomilnacipran

Limit of detection and limit of quantification

A signal–to–noise ratio of 3:1 and 10:1 was considered for calculating LOD and LOQ respectively. The values of LOD and LOQ were given in the table

LOD	1.425µg/ml
LOQ	4.75µg/ml

Table 9: LOD and LOQ

Robustness

Sl.no.	Parameter	Optimized	Used	Retention time (mins)
1	Flow rate	1 ml/min	0.9 ml/min	6.043
			1.1ml/min	5.047
2	Detection wavelength	210 nm	209nm	5.427
			211 nm	5.363

Table 8: Robustness of Levomilnacipran

SUMMARY

1	Column	XBridge™ C18 column 5µ (250 mm x 4.6 mm)
2	Mobile phase	Dipotassium hydrogen phosphate: Methanol (50:50 v/v, pH 6.5)
3	pH	6.5
4	Flow rate	1ml/min
5	Absorption maxima	210 nm
6	Run time	10 mins
7	No. of theoretical plates	4068
8	Retention time	5.29mins
9	Tailing factor	1.38
10	Linearity range	5-30 µg/ml
11	Correlation coefficient (R^2)	0.998
12	Precision (%RSD)	0.90
13	Accuracy (%RSD)	0.46
14	Limit of Detection	1.42µg/ml
15	Limit of Quantification	4.75µg/ml
16	% Recovery	99.86%

CONCLUSION

In addition to positive requirements for analytical methods, the striking advantage of all the developed methods is that they are economical, cheap, and precise. The proposed RP-HPLC method was a suitable technique for the determination of Levomilnacipran. All the parameters analyzing Levomilnacipran met the criteria of ICH guidelines for Method Validation. In the present investigation, we have developed a simple, sensitive, precise, and accurate RP-HPLC method for the quantitative estimation of Levomilnacipran in bulk and pharmaceutical formulations. The recoveries achieved were found good by the method. The HPLC method is more sensitive, precise, and accurate compared to the spectrophotometric methods. The HPLC method developed may be recommended for the routine determination of Levomilnacipran in bulk drug and pharmaceutical formulations.

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