RESEARCH ARTICLE

RP-HPLC Method Validation for Levomilnacipran Estimation in Bulk and Formulation

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Received: 04th September, 2023; Revised: 21st October, 2023; Accepted: 13th November, 2023; Available Online: 25th December, 2023

ABSTRACT

RP-HPLC was used to build sensitive and accurate procedures for establishing and verifying analytical methods for estimating levomilnacipran in bulk and pharmaceutical dose forms. The ratio of methanol to 10 mM dipotassium hydrogen phosphate buffer pH 6.5 in the mobile phase is 50:50 (v/v%). The working standard solution was 20 g/mL. With a flow rate of 1-mL/minutes, an injection volume of 20 µL, a run time of 10 minutes, and a detection wavelength of 215 nm, the analysis was carried out on XBridgeTM C_{18} column 5 (250 x 4.6 mm). Readings of the precision study %RSD were found to be below the 2% limit. With a r^2 of 0.998, it was concluded that the strategy was linear. The results showed that the levomilnacipran LoD and LoQ values were 1.42 and 4.75 µg/mL, respectively. The approach may be used to analyse levomilnacipran bulk and pharmaceutical formulations.

Keywords: Levomilnacipran, Reversed-phase high performance liquid chromatography, Linearity.

International Journal of Pharmaceutical Quality Assurance (2023); DOI: 10.25258/ijpqa.14.4.13

How to cite this article: Boggula N, Bhadru B, More K. RP-HPLC Method Validation for Levomilnacipran Estimation in Bulk and Formulation. International Journal of Pharmaceutical Quality Assurance. 2023;14(4):900-903.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

[(1S,2R)-2-(aminomethyl)-N,N-diethyl-1-phenylcyclopropane-1-carboxamide] is the name of the chemical cmpound (Figure 1). The molecular weight is 264.34 g/mol and the chemical formula is $C_{15}H_{22}N_2O$.[1,2]

Levomilnacipran is an antidepressant. It is mostly employed to treat depression. Levomilnacipran, which functions as a serotonin norepinephrine reuptake inhibitor, is an active enantiomer of milnacipran and as a result, has identical actions and pharmacology.[3-5]

A review of the literature suggests that levomilnacipran can be determined using UV, UPLC, and LC-MS, however, relatively few RP-HPLC procedures have been established. The current study describes a new RP-HPLC technique to determining levomilnacipran in bulk and its formulation.[6]

MATERIALS AND METHODS

Solvents and Reagents

Levomilnacipran was received from Hetero Drugs Ltd. in Hyderabad, Telangana, as a gift sample. I bought 40 mg of the levomilnacipran extended-release pills from my neighborhood drugstore. In the investigation, methanol of HPLC grade, water, and dipotassium hydrogen phosphate (Merck) were utilized.

Solvent Selection

Numerous trials were conducted to determine the best solvent for levomilnacipran dissolution. Based on the drug's solubility, various solvents including HPLC-grade water, methanol, and acetonitrile were explored. Levomilnacipran was sparingly soluble in water but easily soluble in acetonitrile and methanol.

Method Validation[7-11]

The goal of validation was to check that the current approach is appropriate for the purpose specified in ICH standards. The technique was verified to assess the method's system applicability, range, LoD, LoQ, and robustness.

Validation Parameters

Chromatographic conditions

For the analysis, XBridgeTM C_{18} column 5 (250 x 4.6 mm) was employed. With a run time of 10 minutes, the flow rate was set at 1-mL/min. The injection had a 20 µL volume. The detector was adjusted to a 215 nm wavelength.

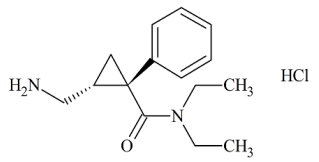

Figure 1: Molecular structure of levomilnacipran

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Accuracy

Methanol was used to produce the solutions for accuracy assessment at 80, 100, and 120%. For 80% accuracy, 32 mg of pure drug was added to 40 mg of the formulation; for 100% accuracy, 40 mg of pure drug was added to 40 mg of the formulation; and for 120% accuracy, 48 mg of pure drug was added to 40 mg of the formulation. The figures of %recovery and %RSD were calculated. Fresh samples were produced for each determination, and accuracy was assessed. Acceptance criterion should be within 98 to 102%.

Linearity

Aliquots of 5, 10, 15, 20, 25, and 30 µg/mL conc. solutions were made using methanol and buffer (50:50). Each of the aforementioned concentrations was divided into six equal dilutions and 20 litres of each concentration were then injected into the HPLC apparatus, where the chromatogram was captured. Each peak's area was measured, and a calibration curve mapping peak area against concentration was made.

Precision

For a batch of 20 g/mL drug solutions that were examined six times on the same day and two days apart, the assay's accuracy was evaluated in terms of intraday and interday fluctuation in the peak area. The graph shows how the peak ratio of the medication solution varies during the day and between days as calculated using the coefficient of variation and determined by the standard deviation to mean ratio multiplied by 100. Requirements for acceptance - %RSD less than 2.

Robustness

Analytical procedures' resilience refers to their ability to remain unaffected by small, deliberate changes in the chromatographic settings. A wavelength result of 1-nm was produced, and it was proved that they were unaffected by a 0.1 mL/min variation in mobile phase flow rate.

Limit of detection and limit of quantification

LoD and LoQ were established using a signal-to-noise ratio-based method in compliance with ICH standards. We compared the signals from chromatograms made with known minimal amounts of analytes to the signals from control samples. Signal-to-noise ratios of 3:1 and 10:1 was used to derive LoD and LoQ, respectively.

RESULTS AND DISCUSSION

Solubility

Levomilnacipran API was freely soluble in methanol. Hence, methanol was selected as the optimized solvent in this method.

Detection of Absorption Maxima by UV Spectroscopy

Separately, weigh and transfer 100 mg of the working standard for levomilnacipran into a 100 mL volumetric flask. Add 100 mL of methanol and sonicate for 10 minutes to dissolve. To achieve a concentration of 100 g/mL, 1-mL of this stock solution (1-mg/mL) was added to a 10 mL volumetric flask that had been filled with methanol to its full 10 mL capacity.

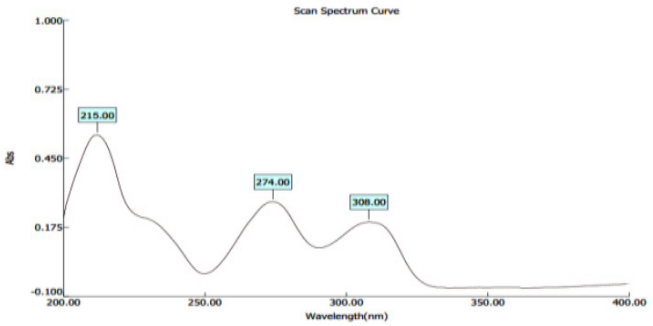

Figure 2: UV spectrum of levomilnacipran

Table 1: Assay

Injection (20 mcg/mL)	Peak area
1	18581355
2	18611533
3	18436743
4	18527401
5	18582923
6	18396594
Average	18522758.17
Standard deviation	87490.05
RSD (%)	0.47

Last but not least, add 10 mL of methanol to 1-mL of the aforementioned solution and carefully mix. The concentration of the usable solution that was so produced was 10 g/mL. Levomilnacipran working standard solutions (10 g/mL) were scanned between 200 and 400 nm using a blank as a reference. The absorption maxima at 215 nm were determined by observing the overlaid spectra, and experiments were carried out at this wavelength by observing the overlaid spectra, and experiments were carried out at this wavelength (Figure 2).

Assay of Formulation

Twenty 40 mg levomilnacipran capsules were weighed and roughly ground. Levomilnacipran powder weighing A 50 mg was added to a 50 mL volumetric flask with 30 mL of methanol. The mixture was sonicated for 20 minutes. The 50 mL capacity was filled with methanol. The contents were filtered via a 0.45 membrane filter. A concentration of 20 g/mL was achieved through additional dilutions. Chromatograms were logged for up to 10 minutes after separate injections of 20 mL each of the test and reference solutions were produced. It was established that the suggested method was accurate and unaffected by the excipients in capsules (Table 1).

Accuracy

The accuracy results were tabulated (Table 2).

Linearity

With methanol and buffer (50:50), aliquots of 5, 10, 15, 20, 25, and 30 were made using the working standard. Each of the aforementioned concentrations was divided into six separate dilutions, from which 20 L of each concentration was fed into

Table 2: Accuracy

Level of percentage recovery	*80%*	*100%*	*120%*
Amount present (mg/tablet)	40	40	40
Amount of 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	80.0	88.23
% Recovery	99.96	100.01	100.26

Table 3: Linearity

Concentration in (µg/mL)	*Area*
5	4349649
10	8285566
15	12834720
20	18367390
25	22329732
30	26871928

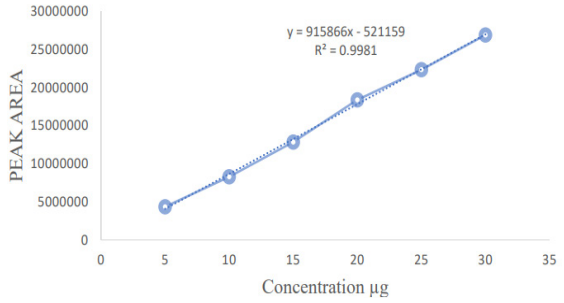

Figure 3: Linearity graph

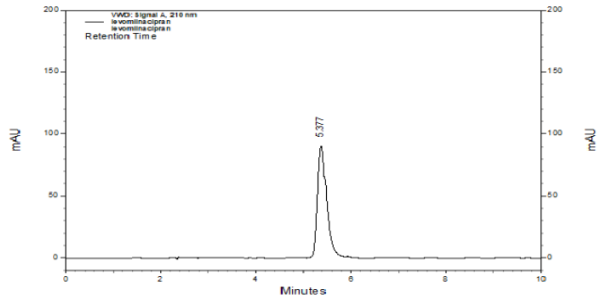

Figure 4: Optimized chromatogram standard

the HPLC apparatus. Then their chromatogram was recorded. Each peak's area was measured, and a calibration curve plotting peak area against concentration was created (Table 3 and Figures 3, 4).

Precision

Batch of 20g/ml drug solutions that were examined six times on the same day and two days apart, the assay's accuracy was evaluated in terms of intraday and interday fluctuation in the peak area. The intraday results were evaluated and tabulated

Table 4: Intraday precision (morning)

Injection (20 mcg/mL)	*Peak area*
I	18444069
II	18331618
III	18310213
IV	18172297
V	18544532
VI	18669938
Average	18412111.17
Standard deviation	178552.39
RSD (%)	0.97

Table 5: Intraday precision (afternoon)

Injection (20 mcg/mL)	*Peak area*
I	18435750
II	18698702
III	18310214
IV	18272296
V	18544531
VI	18669939
Average	18488572.00
Standard deviation	179753.76
RSD (%)	0.97

Table 6: Intraday precision (Day 1)

Injection (20 mcg/mL)	*Peak area*
I	18611787
II	18425770
III	18882973
IV	18809578
V	18568722
VI	18693988
Average	18665469.67
Standard deviation	166484.88
RSD (%)	0.89

Table 7: Intraday precision (Day 2)

Injection (20 mcg/mL)	*Peak area*
I	18611787
II	18425770
III	18882973
IV	18809578
V	18568722
VI	18693988
Average	18665469.67
Standard Deviation	166484.88
RSD (%)	0.80

Table 8: Robustness

Parameter	*Optimized*	*Utilized*	R_t *(min)*
Rate of flow	1 mL/min	0.9 mL/min	6.043
		1.1ml/min	5.047
Wavelength (nm)	215	214	5.427
		216	5.363

Table 9: Limit of detection and limit of quantification

Levomilnacipran	LoD	1.425 µg/mL
	LoQ	4.75 µg/mL

Table 10: Parameters

Column	*XBridge™ C_{18} column 5 µ (250 mm x 4.6 mm)*
Mobile phase	Dipotassium hydrogen phosphate: methanol (50:50 v/v)
pH	6.6
Flow rate	1-mL/min
Run time	10 minutes
Absorption maxima	215 nm
No. of theoretical plates	4068
Retention time	5.29 minutes
Tailing factor	1.38
Linearity	5–30 µg/mL
r^2	0.998
Accuracy (%RSD)	0.46
Precision (%RSD)	0.90
%Recovery	99.86%
LoD	1.425 µg/mL
LoQ	4.75 µg/mL

(Table 4 and 5). The interday resuls were calculated and reported (Table 6 and 7).

Robustness

The robustness result was calculated and tabulated (Table 8).

LoD and LoQ

For determining LoD and LoQ, a signal-to-noise ratio of 3:1 and 10:1 was used, respectively (Table 9 below lists the values for LoD and LoQ).

All the chromatographic conditions and parameters evaluated in the present research work were tabulated (Table 10).

CONCLUSION

In addition to meeting the positive requirements for analytical procedures, all of the created methods were affordable, inexpensive, and precise. The suggested RP-HPLC method was an appropriate methodology for determining levomilnacipran. All of the parameters used to analyse levomilnacipran satisfied the ICH method validation requirements. In this study, we devised a quick, accurate, precise, and sensitive RP-HPLC method for measuring levomilnacipran in pharmaceutical formulations and bulk.

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