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Development and Validation of Zero and First Order Spectrophotometric Method for Determination of Levomilnacipran in Bulk and Formulation

M Akiful Haque, Boggula Narender ,Cherukuri Sravanthi, Burugu Rakshanda Goud, Bukka Sony, Routhu Deepshika, Vasudha Bakshi

Department of Pharmaceutical Chemistry, School of Pharmacy, Anurag Group of Institutions, Venkatapur, Ghatkesar, Telangana, India.

Abstract

Objective: The objective of the present work is to develop a novel, simple and economic method for the quantification of levomilnacipran in bulk materials and in tablet formulation. Further this study is designed to validate the developed methods as per ICH guidelines.

Methods: The quantification process was performed on UV-Spectrometer. The solutions of standard and sample were prepared in distilled water. After suitable dilution, 10µg/mL of levomilnacipran was prepared and scanned in the UV-visible range 400-200nm. In the quantitative determination of the drug carried for zero order at 220nm and first order at 217nm, and linearity range was formed to be 5-25µg/mL (r^2>0.999). Levomilnacipran showed a maximum absorbance at 220nm and 217nm of zero and first order derivative respectively. Area under curve (AUC) of the zero-order spectrum and first order spectrum was recorded between 210-230nm and 217-230nm respectively. For a linearity study, series of dilutions were prepared from standard stock solutions.

Results: The method is valid for the quantification of levomilnacipran over a linearity range of 5-25µg/mL (r^2>0.999). The results of linearity, accuracy, precision, LOD, LOQ, recovery was within acceptable range for both zero and first order derivative.

Conclusion: The developed method is simple, precise, rugged, robust, and economical. The method can be used for routine analysis of levomilnacipran from its tablet formulation. The described methods can be readily utilized for analysis of pharmaceutical formulation.

Key words: Levomilnacipran, derivative-spectrophotometry, zero order spectra, first order derivatives, analytical method validation.

INTRODUCTION

Pharmaceutical analysis is a branch of practical chemistry that involves a series of process for identification, determination, quantification and purification of a substance, separation of the components of a solution or mixture, or determination of structure of chemical compounds. The substance may be a single compound or a mixture of compounds and it may be in any of the dosage form. The substance used as pharmaceuticals are animals, plants, microorganisms, minerals and various synthetic products. Analytical chemistry is the science that seeks ever improved means of measuring the chemical composition of natural and artificial materials. Analytical monitoring of a pharmaceutical product or of specific ingredients within the product is necessary to ensure its safety efficacy throughout all phases of its shelf life. Such monitoring is in accordance with the specifications elaborated during product development. Analytical method validation is the corner stone of process validation without a proven measurement system it is impossible to confirm whether the manufacturing process has done what it purports to do. All new analytical methods developed are validated.

UV radiation originates a great number of biological and chemical processes which in the majority of cases are hazardous for animal and plant systems. The influence of UV radiation on the organism of a human being is mostly expressed by the influence on the eyes, skin and immune system. Long-lasting and prolonged stay in the Sun increases the relativity of aging of the skin and causes the appearance of dangerous skin diseases caused by the surplus of UV radiation.

Spectrophotometric methods of analysis are more economic and simpler, compared to methods such as chromatography and electrophoresis. Under computer-controlled instrumentation, derivative spectrophotometry is acting a very important role in the single or multicomponent analysis of drugs by UV molecular absorption spectrophotometric method. Pharmaceutical research is developing increasingly complex molecules and drug formulations, and each novel and highly selective analytical technique is therefore of much potential interest. Levomilnacipran is a novel serotonin and norepinephrine reuptake inhibitor (SNRI) for the treatment of major depressive disorder. The chemical name is [(1S,2R)-2-(aminomethyl)-N,N-diethyl-1-phenylcyclopropane-1-carboxamide].

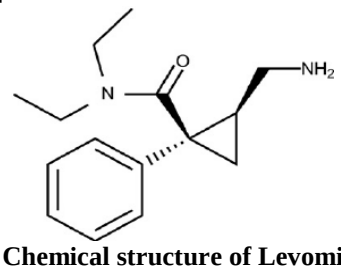

Figure 1: Chemical structure of Levomilnacipran

A literature survey revealed a few methods such as HPLC, stability-indicating LC, and UV-Spectrophotometric methods for the determination of levomilnacipran in bulk

material and in tablets. In the present research work a simple, economical, and rapid spectrophotometric method have been established for the quantification of levomilnacipran in bulk material and in tablets. The developed methods were validated for accuracy, precision, ruggedness, and sensitivity. Accordingly, the objective of this study was to develop and validate the simple spectrophotometric method for the estimation of levomilnacipran in bulk and tablets as per ICH guidelines.

MATERIALS AND METHODS

Instrumentation

Analysis carried out on Schimadzu UV-1800, UV/Vis-Spectrophotometer, a single beam high scanning spectrophotometer (200-400nm) with a photo diode array detector. Digital balance for weighing and sonicator were used for the study.

Materials

The drug sample of levomilnacipran was received as a gift sample from Hetero labs, Hyderabad, Telangana, India. Distilled water was used as solvent throughout the experimentation. A pharmaceutical preparation was purchased from the local pharmacy.

Analytical method development

Method-1: Development of zero order spectroscopic method

Standard stock solution

To prepare standard stock solution of levomilnacipran (1000 µg/ml), 100mg of drug was placed in 100ml of volumetric flask and dissolved in 25ml of distil water and the volume was made up to the mark with distil water. 10ml of the solution was diluted up to 100ml with distil water to produce final stock solution of 100 µg/ml of levomilnacipran.

Sample preparation

Twenty tablets were taken, powdered and powder weight equivalent to 500mg of levomilnacipran was accurately taken and transferred to 50ml of volumetric flask. 20ml of distil water was added to same and sonicated for 30 mins. The flask was shaken and the volume was diluted to the mark with same mixture. The above solution was filtered using whatman filter paper. Appropriate volume of the aliquot was transferred to a 50ml volumetric flask and the volume was made up to the mark with distil water. The spectra was recorded and then measured at 220nm for levomilnacipran. The overlain spectra and calibration curve are shown in Figure 2 and 3.

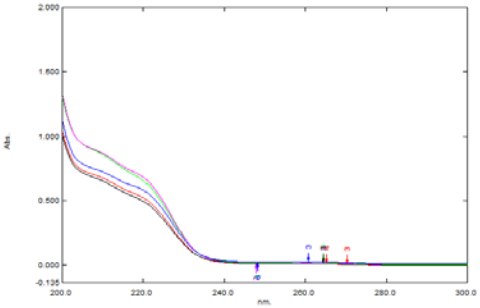

Figure 2: Overlay spectrum of levomilnacipran at 220nm (zero order derivative)

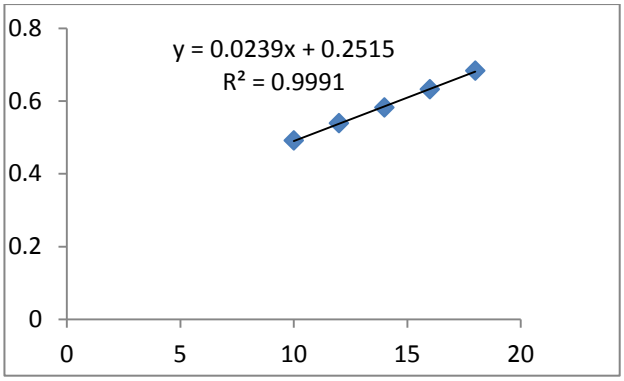

Figure 3: Calibration graph of levomilnacipran at 220 nm (zero order derivative)

Method-2: Development of first order derivative spectroscopic method

Standard stock solution

To prepare standard stock solution of levomilnacipran (1000 µg/ml), 100mg of drug was placed in 100ml of volumetric flask and dissolved in 25ml of distil water and the volume was made up to the mark with distil water. 10ml of the solution was diluted up to 100ml with water to produce final stock solution of 100 µg/ml of levomilnacipran.

Sample preparation

Twenty tablets were taken, powdered and powder weight equivalent to 1000mg of levomilnacipran was accurately taken and transferred to 50 ml of volumetric flask. 20ml of water was added to same and sonicated for 30 mins. The flask was shaken and the volume was diluted to the mark with same mixture. The above solution was filtered using whatman filter paper. Appropriate volume of the aliquot was transferred to a 50 ml volumetric flask and the volume was made up to the mark with water. The first derivative spectra was recorded and then measured at 217nm for levomilnacipran. The derivative spectra and calibration curve are shown in Figure 4 and 5.

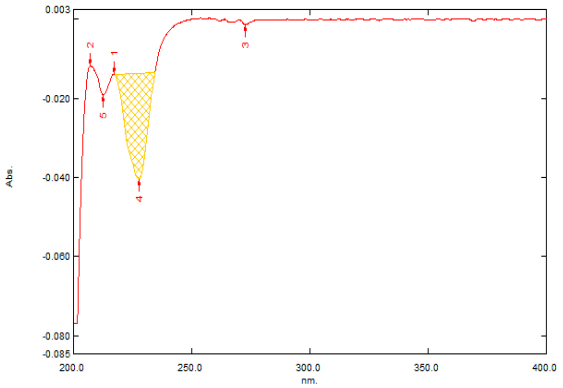

Figure 4: Derivative spectra for levomilnacipran

Linearity studies

Five points calibration curve were obtained in a concentration range from 5-25 µg/ml for levomilnacipran. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was noted.

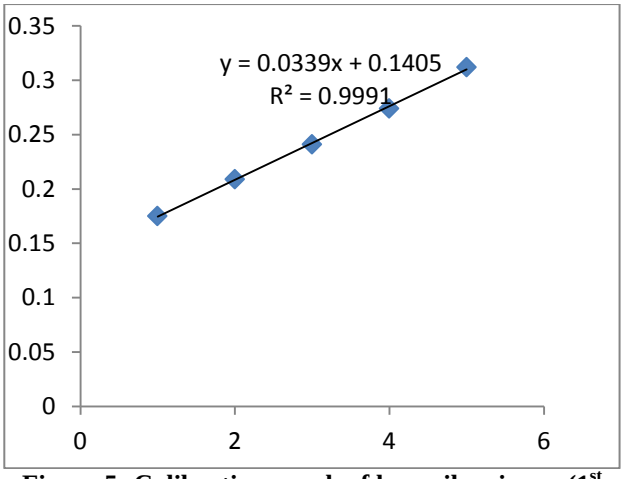

Figure 5: Calibration graph of levomilnacipran (1st order derivative)

Precision

Precision is determined by intraday and interday precision. Intra-day precision was determined by analyzing the 10, 12, 14, 16 and 18µg/ml for three times in the same day. Inter-day precision was determined by analyzing the same concentration of the solutions daily for three days.

Recovery

To the pre-analyzed sample solutions 10µg/ml of levomilnacipran a known amount of standard stock solutions were added at different levels 80%, 100% and 120%. The solutions were reanalyzed by proposed method.

Accuracy

To determine the accuracy, studies were carried out three different levels i.e. 80%, 100% and 120%. To the pre-analyzed sample solution a known amount standard drug solution was added at three different levels, absorbance was recorded.

Ruggedness and robustness

Ruggedness of the proposed method is determined by analysis the aliquots from homogenous slot by two analyst using operational and environmental conditions.

LOD and LOQ

Several approaches for determining the LOD & LOQ are possible depending on whether the procedure is non instrumental or instrumental.

RESULTS AND DISCUSSION

The method was validated according to International Conference on Harmonization (ICH guideline) absolute guidelines for validation of analytical method for the determination of linearity, precision and accuracy. Overlay spectrum of levomilnacipran of zero order derivative was shown in Figure 2. Precision of the method was determined by adding known amounts of pure drug (80, 100 and 120%) in triplicate. It was found that the differences of <5.0 % for intra & inter day reflect the precision of the method. The observation of percentage CV <10 for intra & inter day measurements also indicates a high degree of precision. The results of precision were illustrated in Table 2 & 3. The linearity concentration ranges from 5-25 µg/ml (r^2>0.999). The regression curve of levomilnacipran for zero and first order method are reported in Figure 3 & 5. In UV-Spectroscopic method, the zero and first order spectra were utilized for developing the equations for analysis. Levomilnacipran showed maximum absorbance at 220nm and 217nm for zero and first order derivative spectroscopy respectively. The normal spectra were derivatized into first order derivative, using UV software of instrument, where $\Delta\lambda$ = 2. The recovery results gave good recovery <101% and results are shown in Table 4. LOD & LOQ results are represented in Table 5. The results of ruggedness & robustness are shown in Table 6 & 7. For both the methods the results was found to be satisfactory.

Linearity study of levomilnacipran

Present study showed the applicability of multivariate linear regression move towards to the UV data obtained at different wavelengths for the calibration and tablet analysis. Statistically, the use of vast number of data calculated for a sample analysis makes the results satisfactory. The data obtained for the estimation of levomilnacipran bulk and marketed formulation proved the high level accuracy and precision after calibration. The developed method was successfully validated for Method 1 and 2.

Table 1: Linearity studies of levomilnacipran (zero &first order derivative)

Method 1		Method 2	
Concentration	**Absorbance**	**Concentration**	**Absorbance**
10 µg/ml	0.175	10 µg/ml	0.491
12 µg/ml	0.209	12 µg/ml	0.539
14 µg/ml	0.241	14 µg/ml	0.582
16 µg/ml	0.274	16 µg/ml	0.632
18 µg/ml	0.312	18 µg/ml	0.682

Table 2: Precision studies of levomilnacipran (zero &first order derivative)

Method 1					
Intraday			Interday		
Conc. (µg/ml)	**Standard deviation**	**Relative std. deviation**	**Conc. (µg/ml)**	**Standard deviation**	**Relative std. deviation**
10	`0.00178	0.36%	10	0.00151	0.30%
12	0.00158	0.29%	12	0.00134	0.24%
14	0.00130	0.22%	14	0.00151	0.26%
16	0.00178	0.28%	16	0.00130	0.20%
18	0.00178	0.26%	18	0.00151	0.22%

Table 3: Intraday and Interday precision of levomilnacipran (zero order derivative)

Method 2					
Intraday			Interday		
Conc. (µg/ml)	**Standard deviation**	**Relative std. deviation**	**Conc. (µg/ml)**	**Standard deviation**	**Relative std. deviation**
10	0.00044	0.25%	10	0.00083	0.47%
12	0.00054	0.25%	12	0.00083	0.39%
14	0.00054	0.22%	14	0.00083	0.34%
16	0.00054	0.19%	16	0.00083	0.30%
18	0.00044	0.14%	18	0.00083	0.20%

Table 4: % Recovery of levomilnacipran (zero & first order derivative)

Method 1			Method 2		
Amount of drug added	**Absorbance**	**Amount recovered**	**Amount of drug added**	**Absorbance**	**Amount recovered**
8 µg/ml	0.883	101%	8 µg/ml	0.315	100%
10 µg/ml	0.982	99.9%	10 µg/ml	0.36	101%
12 µg/ml	1.080	100%	12 µg/ml	0.21	99.9%

Table 5: LOD & LOQ of levomilnacipran (zero & first order derivatives)

METHOD 1		METHOD 2	
LOD	**LOQ**	**LOD**	**LOQ**
0.25	0.77	0.044	0.133

Table 6: % Ruggedness of levomilnacipran (zero & first order derivative)

	Parameter	**Method 1**		**Method 2**	
		Standard deviation	**Relative standard deviation**	**Standard deviation**	**Relative standard deviation**
Conc. 10µg/ml	Analysist 1	0.00151	0.30%	0.00044	0.25%
	Analysist 2	0.00151	0.30%	0.00044	0.25%
	Instrument 1	0.00151	0.30%	0.00044	0.25%
	Instrument 2	0.00151	0.30%	0.00044	0.25%
	Lab 1	0.00151	0.30%	0.00044	0.25%
	Lab 2	0.00151	0.30%	0.00044	0.25%

Table 7: Robustness of levomilnacipran (zero & first order derivative)

Method 1			Method 2		
Wave length	**Standard deviation**	**Relative standard deviation**	**Wave length**	**Standard deviation**	**Relative standard deviation**
218nm	0.00178	0.36%	218	0.00044	0.25%
222nm	0.00083	0.16%	222	0.00054	0.30%

CONCLUSION

The present analytical method for the quantification of levomilnacipran was validated as per ICH Q2 (R1) guideline and it meets to specific acceptance criteria. The direct UV method development for the analysis of levomilnacipran can be applied for the routine analysis of formulation. The UV-Spectroscopic method was found to be rapid, specific, precise, accurate and cost-effective quality control tool for the routine analysis of levomilnacipran (API) in bulk and tablet dosage form. The present analytical method can be used for its intended purpose.

In conclusion, an enormously, unique, reproducible, UV-Spectrometers approach became advanced based on procedure for the estimation of levomilnacipran research. The linearity range was 5-25µg/mL (r^2>0.999). The conditions of the method have been optimized to be able to enhance the sensitivity and robustness of the technique. A summary of validation parameters and the effects are provided above. The method can be used for routine analysis of levomilnacipran from its tablet formulation. Results also prove that the developed methods can be successfully applied for a regular analysis and quantitative control of drug.

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Author's Contribution

All the authors contributed equally.

Conflict Of Interest

Author declares that there is no conflict of interest to disclose.

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