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Development and Validation of Spectrophotometric method for estimation of Levomilnacipran in Bulk and Pharmaceutical dosage form by Area Under Curve

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ABSTRACT: Background: A simple, rapid, accurate and economical Area Under Curve (AUC) method has been developed for estimation of levomilnacipran from its bulk and pharmaceutical formulation. **Aim:** 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. **Method:** The quantification process was performed on UV-Spectrometer. The solutions of standard and sample were prepared in distilled water. After suitable dilution, 100 µg/ml of levomilnacipran was prepared and scanned in the UV-Visible range 400 to 200 nm. In the quantitative determination of the drug carried for area under curve method at 220 to 230 nm and linearity range was formed to be 5 to 25 µg/ml. For a linearity study, series of dilutions were prepared from stock solutions. **Results:** The method is valid for the quantification of levomilnacipran over a linearity range of 5 to 25 µg/ml (r^2>0.999). The results of linearity, accuracy, precision, LOD, LOQ, recovery were within acceptable range for area under curve method. **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.

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INTRODUCTION:

Pharmaceutical analysis is a branch of practical chemistry that involves a series of processes 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 [1,2]. The substance may be a single compound or a mixture of compounds and it may be in any of the dosage forms. 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 [3,4]. 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 cornerstone 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 [5-8]. 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 [9-11].

The AUC (Area Under Curve) method is applicable where there is no sharp peak or when broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ_1 and λ_2. Area calculation processing item calculates the area bounded by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area has to be calculated. This wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. The spectrum obtained from the first order derivative was used to calculate AUC. The calibration curve was constructed by plotting concentration (5 to 25 µg/ml) versus AUC [12-14].

Levomilnacipran (brand name Fetzima) is an antidepressant which was approved in the United States in 2013 for the treatment of major depressive disorder (MDD) in adults. It is the levorotatory enantiomer of milnacipran, and has similar effects and pharmacology, acting as a serotonin-norepinephrine reuptake inhibitor (SNRI). The chemical name is [(1S,2R)-2-(aminomethyl)-N,N-diethyl-1-phenylcyclopropane-1-carboxamide] (Fig 1) [15-19]. Molecular formula is $C_{15}H_{22}N_2O$ and Molecular weight is 264.34 g/mol.

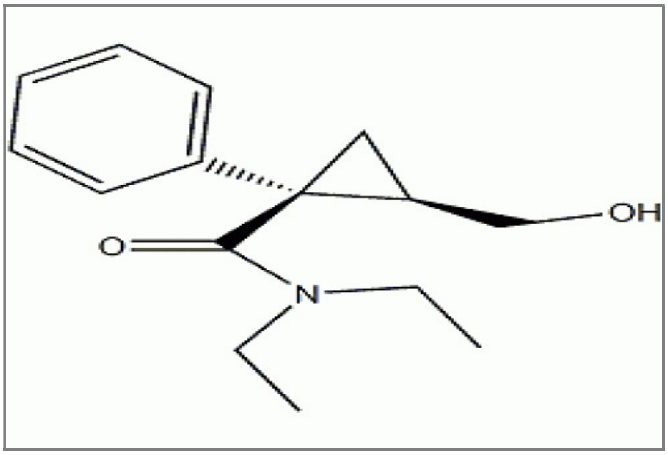

Fig 1. Chemical structure of levomilnacipran.

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 has 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 Shimadzu UV-1800, UV/Vis-Spectrophotometer, and a single beam high scanning spectrophotometer (200 to 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 [20,21]:

Preparation of standard stock solutions of levomilnacipran:

Accurately 100 mg of levomilnacipran was weighed and transferred into 100 ml volumetric flask. It was dissolved in distilled water and was shaken manually for

10 min. The volume was made up to the mark with the same solvent to obtain final strength 1000 µg/ml.

Preparation of Solution 2:

From the stock solutions, 10 ml of levomilnacipran was transferred to 100 ml volumetric flask and the volume was adjusted to the mark with the same solvent to obtain strength 100 µg/ml.

Selection of range:

Standard solution scans in UV-spectrophotometer between 200 nm to 400 nm on spectrum mode, using diluents as a blank. Levomilnacipran shows the range at 220 to 230 nm. The proposed analytical method is simple, accurate and reproducible. The spectra are shown in Fig 2.

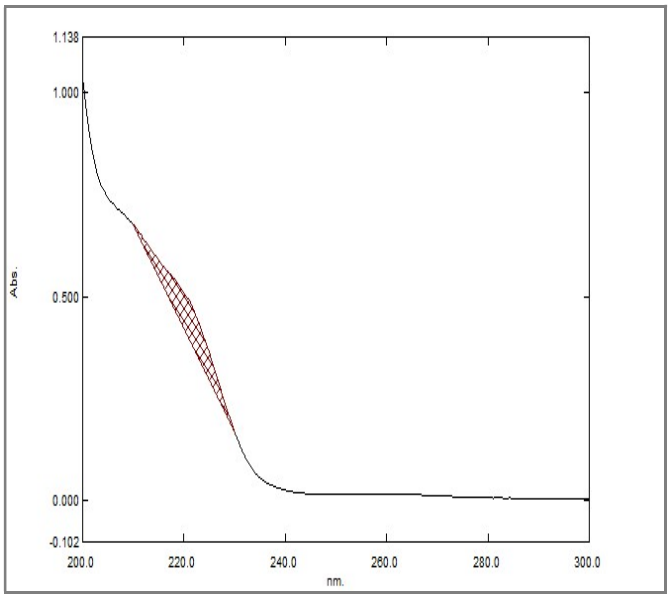

Fig 2. Selection of wavelength AUC range (220 to 230 nm).

Method - Area under curve (AUC):

The AUC (area under curve) method is applicable where there is no sharp peak or when broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ_1 and λ_2. Area calculation processing item calculates the area bounded by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area has to be calculated. This wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. The spectrum obtained from the first order derivative was used to calculate AUC. The calibration curve was constructed by plotting concentration (5 to 25 µg/mL) versus AUC.

Validation of the method:

The method was validated in terms of linearity, accuracy, precision, repeatability and ruggedness.

***Linearity study*:**

Different aliquots of levomilnacipran in range 0.5, 1.0, 1.5, 2.0 and 2.5 ml were transferred in to series of each 10 ml volumetric flasks and the volume was made up to the mark with water to get concentrations 5, 10, 15, 20 and 25 µg/ml respectively. The solutions were scanned on a spectrophotometer in the UV range 200 to 400 nm. The two wavelengths 220 and 230 nm were selected for the determination of area under curve (AUC). The calibration plot was constructed as an area under curve v/s concentration.

***Accuracy (% Recovery)*:**

To the pre analysed sample solutions, a known amount of standard stock solution was added at different levels i.e. 80, 100 and 120 %. The solutions were reanalysed by the proposed method.

***Precision*:**

Precision of the method was studied as intraday and inter-day variations. Intra-day precision was determined by analysing the 10, 12, 14, 16 and 18 µg/ml of levomilnacipran solutions for three times in the same day. Inter-day precision was determined by analysing the 10, 12, 14, 16 and 18 µg/ml of levomilnacipran solutions daily for three days over the period of week.

***LOD and LOQ (Sensitivity)*:**

The sensitivity of measurements of levomilnacipran by the use of the proposed method was estimated in terms of the Limit of Quantification (LOQ) and Limit of Detection (LOD). The LOD & LOQ were calculated using following equations;

$LOD = 3.3 \times N/B$ …..(1)

$LOQ = 10 \times N/B$ …..(2)

Where, N is standard deviation of the peak areas of the drugs (n=3), taken as a measure of noise, and B is the slope of the corresponding calibration curve.

***Repeatability*:**

Repeatability was determined by analysing 10 µg/ml concentration of levomilnacipran solution for six times.

***Ruggedness*:**

Ruggedness of the proposed method is determined for 10 µg/ml concentration of levomilnacipran by analysis of dilution from homogenous slot by two analysts using same operational and environmental conditions.

Determination of levomilnacipran in bulk:

Accurately weighed 10 mg of levomilnacipran was transferred to a 100 ml volumetric flask and 50 ml water was added. After shaking for 2 min, the mixture was diluted up to mark with water. From the stock solution correct dilution was taken in such a way that the final concentration is 10 µg/ml. The concentrations of the drug were calculated from linear regression equations. The resulting solution was scanned on a spectrophotometer in the UV range 200 to 400 nm. The spectrum was recorded between 220 to 230 nm.

Application of proposed method for pharmaceutical formulation:

For analysis of commercial formulation two tablets of 5 mg of levomilnacipran were transferred to a 100 ml volumetric flask and 50 ml water was added. After ultrasonic vibration for 15 min, the mixture was diluted up to mark with water. The whole solution filtered using whatman filter paper no. 42. From filtrate correct dilution was taken in such a way that the final concentration is 10 µg/ml. The concentrations of the drug were calculated from linear regression equations. The resulting solution was scanned on a spectrophotometer in the UV range 200 to 400 nm. The spectrum was recorded between 220 to 230 nm.

RESULTS AND DISCUSSION:

Method validation:

The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the standard procedure given in the experiment.

Linearity studies:

The linear regression data for the calibration curves showed good linear relationship over the concentration range 5 to 25 µg/ml for levomilnacipran (Fig 3). Linear regression equation was found to be Y = 0.0108 + 0.0051 (r^2 = 0.999). The result was expressed in Table 1.

Table 1. Linearity study of levomilnacipran.

Concentration (µg/ml)	Absorbance, Mean ± SD (n=6)	% RSD
5	0.1373 ± 0.0015	1.1236
10	0.2648 ± 0.0024	0.9353
15	0.3893 ± 0.0038	0.9875
20	0.5245 ± 0.0015	0.2915
25	0.6553 ± 0.0037	0.5666

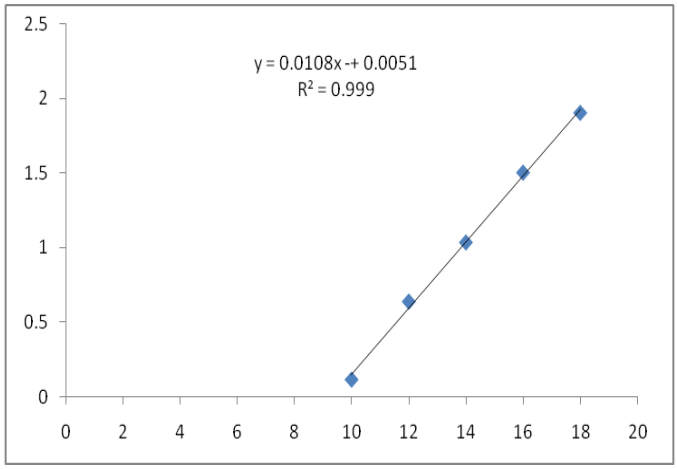

Fig 3. Calibration graph of levomilnacipran.

Accuracy:

The solutions were reanalysed by proposed method; results of recovery studies are reported in Table 2, which showed that the % amount was found in the range of 97.6 to 100.52 % with % RSD < 2.

Table 2. The percentage Recovery of levomilnacipran.

Drug	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovered	% RSD
Levo-milnacipran	8	28.1265	97.6616	1.6569
	10	36.2098	100.5830	1.5214
	12	43.2098	100.0229	0.2511

Precision:

The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). This result shows reproducibility of the assay. The % RSD values found to be less than 2, so that indicates this method is precise for the determination of both the drugs in formulation (Table 3).

Table 3. Results of precision studies.

Conc. (µg/ml)	Intra-day		Inter-day	
	Standard deviation	% RSD	Standard deviation	% RSD
10	0.00044	0.38 %	0.00099	0.86 %
12	0.00054	0.08 %	0.00100	0.15 %
14	0.00054	0.05 %	0.00099	0.09 %
16	0.01303	0.91 %	0.01788	1.24 %
18	0.05477	1.91 %	0.03346	1.84 %

Sensitivity:

The linearity equation was found to be Y = 0.0108 + 0.0051 (r^2 = 0.999). The LOQ and LOD for levomilnacipran were found to be 0.019 and 0.016 µg respectively (Table 4).

Table 4. Sensitivity studies.

LOD (µg/ml)	LOQ (µg/ml)
0.019	0.016

Repeatability:

Repeatability was determined by analysing 10 µg/ml concentration of levomilnacipran solution for six times and the % amount found was between 97 to 101 % with % RSD less than 2 (Table 5).

Table 5. Repeatability studies.

Compo-nent	Amount taken (µg/ml) (n=3)	Amount Found[a] (%)	% RSD
Levomilna-cipran	10	48.12 ± 0.17	0.36

Data is presented as mean ± standard deviation.

Ruggedness:

Peak area was measured for the same concentration solutions, six times. The results are in the acceptable range for both the drugs. The results are given in Table 6. The result showed that the % RSD was less than 2 %.

Table 6. Ruggedness study.

Compo-nent	Amount taken (µg/ml) (n=3)	Amount found (%)[a]	
		Analyst I ± SD	Analyst II ± SD
Levomilna cipran	10	99.4213 ± 0.2249	99.9357 ± 0.2952

Data is presented as mean ± standard deviation.

Determination of levomilnacipran in bulk:

The concentrations of the drug were calculated from linear regression equations. The % amount found and it was in the range of 99.00 to 101.00 % illustrated in Table 7.

Application of proposed method for pharmaceutical formulation:

The spectrum was recorded at 289 nm. The concentrations of the drug were calculated from a linear regression equation. The % amount was found in the range of 98.00 to 100.00 %, shown in Table 8.

Table 7. Analysis of levomilnacipran in bulk.

Conc. (µg/ml)	Amount found (µg)	Amount found (%)
10	82.7037	98.4567
	84.5925	100.7055
	83.3240	99.1953
	84.6111	100.7275
	83.6944	99.6362
	83.3888	99.2724
Mean ± SD	83.7191 ± 0.7556	99.6656 ± 0.8996
% RSD	0.9026	0.9026

Data is presented as mean ± standard deviation.

Table 8: Analysis of levomilnacipran in formulation.

Conc. (µg/ml)	Amount found (µg)	Amount found (%)
10	82.5925	98.3245
	82.3518	98.0379
	83.9166	99.9001
	83.9722	99.9669
	83.1388	98.9748
	83.1944	99.0410
Mean ± SD	83.1944 ± 0.6635	99.0410 ± 0.7899
% RSD	0.7976	0.7976

Data is presented as mean ± standard deviation.

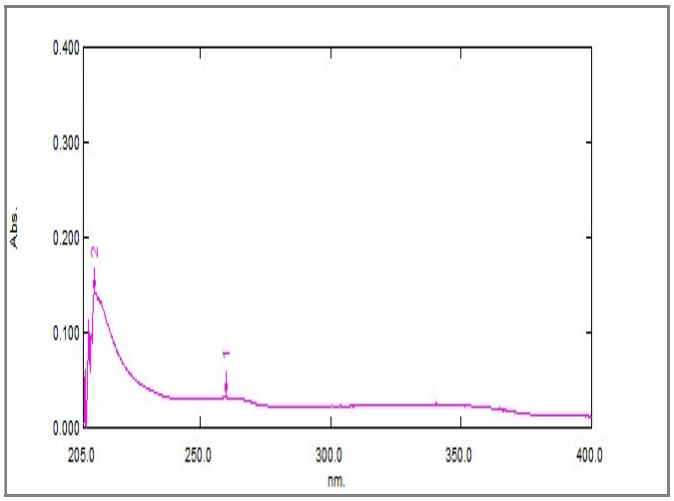

Fig 4. The spectra data of levomilnacipran formulation.

CONCLUSION:

In conclusion, an enormously, unique, reproducible, UV-Spectrometers approach became advanced based on procedure for the estimation of levomilnacipran research. The method was validated according to the International Conference on Harmonization (ICH guideline) absolute guidelines for validation of analytical methods for the determination of linearity,

precision and accuracy. The validation procedure confirms that this is an appropriate technique for their quantification in the formulation.

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 drugs. However, it can serve as an alternative where advanced instruments (e.g. HPLC) are not available for routine analysis.

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