

Development and Validation of UV Spectrophotometric Method for Determination of Milnacipran in bulk and Pharmaceutical Dosage Form

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Abstract

Milnacipran is an antidepressant drug belonging to the class of serotonin and noradrenaline reuptake inhibitors. A new, rapid, simple and sensitive spectrophotometric method has been developed for the determination of milnacipran in bulk and pharmaceutical formulations. The linearity was observed in the concentration range of 2-45 $\mu\text{g mL}^{-1}$. The method is based on spectrophotometric determination. Absorbance of milnacipran was determined at 220 nm wavelength. The method was validated in terms of accuracy and precision (intra and interday variations). This method is extended to pharmaceutical preparations. Results of the analysis were validated statistically and by recovery studies. The proposed method was found accurate, reproducible and economical for the routine analysis of milnacipran in bulk and pharmaceutical formulation.

Keywords:

Milnacipran; Spectrophotometric analysis; Validation

1. Introduction

Milnacipran ((Z)-1-diethylaminocarbonyl-2-aminomethyl-1-phenyl-cyclopropane hydrochloride, Ixel®, Toledomin®, Dalcipran®) is an antidepressant synthesized, developed and marketed by Pierre Fabre Medicament. The drug, which has no affinity for post-synaptic neurotransmitter receptors [1], was selected from a family of 1-aryl-2-aminomethyl cyclopropanecarboxylic acid derivatives [2] for its potent inhibition of both noradrenaline and serotonin reuptake [3,4]. In the treatment of major depression, milnacipran has achieved a similar efficacy to tricyclic antidepressants and a similar tolerability to selective serotonin reuptake inhibitors [5].

Several studies on humans and animals are necessary for the development of a new chemical entity in order to gather extensive knowledge on its pharmacokinetics and metabolism. Therefore, a robust and reliable bioanalytical method is of major importance to allow relevant comparison between studies. The availability of a low cost bioanalytical method, easy to transfer and to set up, represents an advantage in therapeutic drug monitoring when required (control of compliance, overdose) [6].

Milnacipran is not official in any pharmacopoeia. Literature review reveals, there is no report of UV-Visible spectrophotometric method for its estimation. Only few analytical techniques including high performance liquid chromatography (HPLC) with fluorescence

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detection in human plasma and few chiral HPLC methods are available [7,8]. Therefore, an attempt was made to develop a simple spectrophotometric method for the estimation of the present drug in bulk and pharmaceutical formulations.

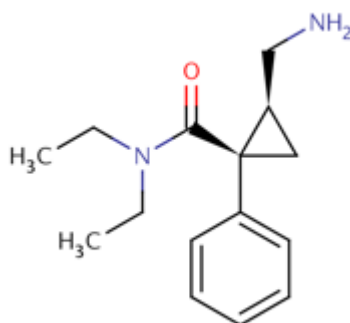


Fig.1. Chemical Structure of Milnacipran

2. Experimental

2.1. Materials

Pharmaceutical grade Milnacipran was obtained from Torrent Research Centre (Gandhinagar, India). Distilled water was used in all stages. Two brands of capsules, MILBORN-25 (Sun Phrama. Ltd, Gujarat, India) and MILNACE 50 (Torrent Research Centre (Gujarat, India) were procured from the local market.

2.2. Apparatus

Spectrophotometric analysis was performed on a Shimadzu UV-1800 spectrophotometer, with a 1.00 cm quartz cells. The instrument settings were optimized to produce a spectrum with about 80% full-scale deflection and acceptable noise level. Each spectrum was recorded in triplicate. For each replicate measurement the cell was refilled with fresh solution.

2.3. Methods

2.3.1. Preparation of Milnacipran standard solutions

A stock solution containing $100 \mu\text{g mL}^{-1}$ of milnacipran was prepared by dissolving 10 mg of milnacipran in distilled water, then transferring into a 100 mL volumetric flask and diluted up to the mark with distilled water. All measurements were made at room temperature. The standard solutions were prepared by the proper dilutions of the stock standard solution with distilled water to reach concentration range of $2\text{-}45 \mu\text{g mL}^{-1}$. The determination was conducted in triplicate.

2.3.2. Preparation of sample solution

A powder (55.10 mg) equivalent to 10 mg of drug was transferred to 100 mL volumetric flask. The content was mixed with 50 mL of distilled water. The mixture was sonicated for 20 min. This solution was filtered through the whatman filter paper No.41 and the filtrate and washings were combined and diluted to the 100 mL with distilled water to get solution having milnacipran $100 \mu\text{g mL}^{-1}$.

2.3.3. Preparation of Calibration Curve

The standard stock solution of milnacipran was scanned in the wavelength range of 200 nm to 400 nm against distilled water as a blank. A calibration curve was constructed over a concentration range 2-45 $\mu\text{g mL}^{-1}$. Absorbance of each solution was measured at the wavelength of 220 nm. Calibration curve was constructed for milnacipran by plotting absorbance versus concentration at 220 nm wavelength. The determination was conducted in triplicate.

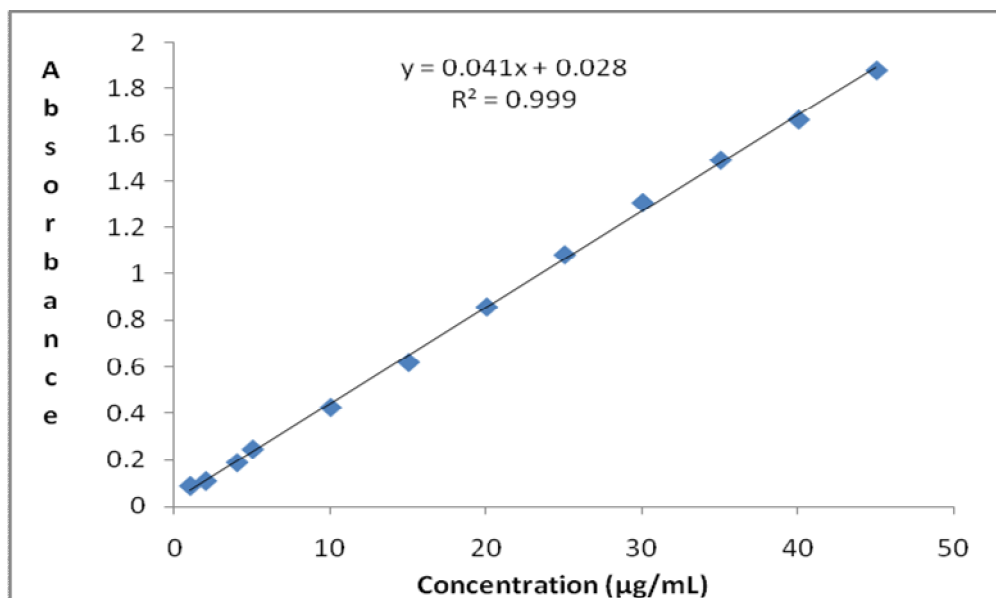


Fig. 2 (a): Calibration Curve of Milnacipran in Distilled water

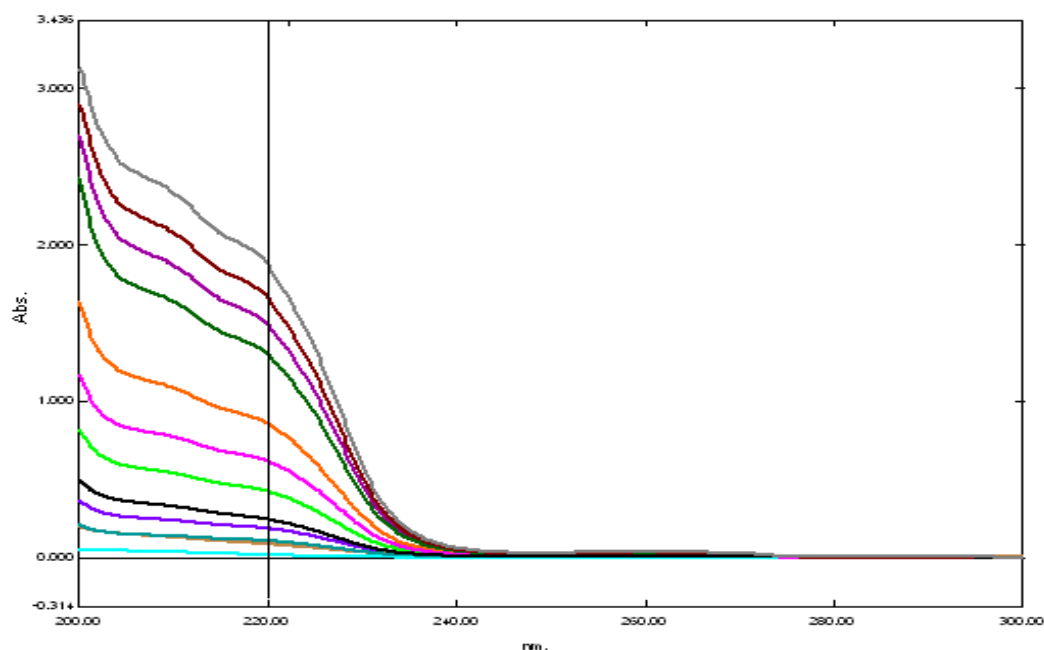


Fig. 2 (b): Overlain Spectrum of Milnacipran in Distilled water ($2-45 \mu\text{g mL}^{-1}$)

2.4. Validation of Method

The method was validated with respect to linearity, accuracy, precision, limit of detection (LOD) and limit of quantitation (LOQ). [9-11]

2.4.1. Linearity

To establish linearity of the proposed method, ten separate series of solutions of Milnacipran (2–45 $\mu\text{g mL}^{-1}$ in distilled water) were prepared from the stock solutions and analyzed. Least square regression analysis was performed on the obtained data.

2.4.2. Accuracy

The accuracy of the method is the closeness of the measured value to the true value for the sample. To determine the accuracy of the proposed method, different levels of drug concentrations lower concentration (LC, 80%), intermediate concentration (IC, 100%) and higher concentration (HC, 120%) were prepared from independent stock solutions and analyzed ($n = 10$). Accuracy was assessed as the percentage relative error and mean % recovery (Table 1). To provide an additional support to the accuracy of the developed assay method, a standard addition method was employed, which involved the addition of different concentrations of pure drug (10, 20 and 30 $\mu\text{g mL}^{-1}$) to a known preanalyzed formulation sample and the total concentration was determined using the proposed method ($n = 10$). The % recovery of the added pure drug was calculated as $\% \text{ recovery} = [(C_t - C_s)/C_a] \times 100$, where C_t is the total drug concentration measured after standard addition; C_s , drug concentration in the formulation sample; C_a , drug concentration added to formulation (Table 1).

Table 1. Recovery studies of Milnacipran capsules ($n=3$)

Label Claim (mg)	Amount added (%)	Recovery (%) \pm S.D	%R.S.D
25	80	99.33 \pm 0.0033	0.0032
	100	99.96 \pm 0.0081	0.0080
	120	100.14. \pm 0.0068	0.0068

2.4.3. Precision

Repeatability was determined by using different levels of drug concentrations (same concentration levels taken in accuracy study), prepared from independent stock solutions and analyzed ($n=10$) (Table 2). Inter-day and intra-day variations were studied to determine intermediate precision of the proposed analytical method. Different levels of drug concentrations in triplicates were prepared three different times in a day and studied for intra-day variation. The same procedure was followed for three different days to study inter-day variation ($n = 10$). The percent relative standard deviation (% R.S.D.) of the predicted concentrations from the regression equation was taken as precision (Table 2). Precision studies were also carried out using the real samples of Milnacipran capsule in a similar way to standard solution to prove the usefulness of the method.

2.4.4. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD ($k=3.3$) and LOQ ($k=10$) of the method was established according to ICH definitions ($C1=k*So/S$, where $C1$ is LOD or LOQ, So is the mean standard deviation of blank determination, S is the slope of standard curve and k is the constant related to confidence interval). LOD and LOQ of method are reported in Table 2.

3. Results and Discussion

The development of spectrophotometry methods for the determination of drugs has increased considerable in recent years because of their importance in pharmaceutical analysis.

Based on the experimental data the standard calibration curve was plotted (Fig.2 (a)). The absorbance range was found to be 0.107-1.879 (Fig. 2(b)). The content of drug was calculated from the equation $y = 0.041x + 0.028$. These solutions obeyed Beer-Lambert's law in concentration range of 2-45 $\mu\text{g mL}^{-1}$ with R^2 value of 0.999. The assays were validated by means of ANOVA (Analysis of variance), as described in official literature [12]. This developed method presented no parallelism deviation and no linearity deviation ($P < 0.05$). The reproducibility of the proposed method was determined by performing capsule assay at different time intervals on same day (Intra-day assay precision) and on three different days (Inter-day precision). Result of intra-day and inter-day precision is expressed in % RSD. Percent RSD for Intraday assay precision was found to be 0.0504. Inter-day assay precision was found to be 0.0905. According to the equation, the LOD and LOQ were found to be 0.27 and 0.82 $\mu\text{g/mL}$, respectively. This data shows that this method is sensitive for the determination of milnacipran. To ascertain the accuracy of proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%). Percent recovery for milnacipran, by the proposed method was found in the range of 99.33 % to 100.14 %. Repeatability is based on the results of the method operating over short time interval under same conditions. The low RSD values of intra-day precision (Table 2), recovery (Table 1), and pharmaceutical preparations (Table 3) showed high repeatability.

Table.2. Optical characteristics of Milnacipran

Parameters	Values
λ_{max} , nm	220
Beer's law limit, $\mu\text{g mL}^{-1}$	2-45
Molar absorptivity, $\text{L/mol}\times\text{cm}$	2.049×10^4
Regression equation	$y = 0.041x + 0.028$
Slope \pm S.D	0.041 ± 0.00014
Intercept \pm S.D	0.028 ± 0.0027
Correlation coefficient (r^2)	0.999
Limit of Detection (LOD), $\mu\text{g mL}^{-1}$	0.27
Limit of Quantitation (LOQ), $\mu\text{g mL}^{-1}$	0.82
Intra day precision (% R.S.D)	0.0504
Inter day precision (% R.S.D)	0.0905

Table 3. Analysis of Milnacipran capsules

Brand name	Label claim (mg)	% Assay \pm S.D	%R.S.D
MILBORN-25	25	99.76 ± 0.0087	0.0087
MILNACE-50	50	99.54 ± 0.0079	0.0080

4. Conclusion

UV spectrophotometric method developed for milnacipran hydrochloride is simple, accurate, sensitive, rapid and economic and it can be conveniently employed for the routine analysis and the quality control of milnacipran hydrochloride in pharmaceutical dosage forms.

The method was suitable to determine concentrations in the range 2-45 $\mu\text{g mL}^{-1}$. The limits of detection and quantitation for milnacipran hydrochloride with a lower concentration were 0.27 and 0.82 $\mu\text{g mL}^{-1}$ respectively, which are under the lowest expected concentrations in the sample. The sample recovery from the formulation was in good agreement with its respective label claim.

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References

1. Assie M B, Charveron M, Palmier C, Puozzo C, Moret C and Briley M (1992) Effects of prolonged administration of milnacipran, a new antidepressant, on receptors and monoamine uptake in the brain of the rat. *Neuropharmacology* 31(2):149-55
2. Bonnaud B, Cousse H, Mouzon G, Briley M, Stenger A, Fauran F and Couzinier JP (1987) 1-Aryl-2-(aminomethyl) cyclopropanecarboxylic acid derivatives. A new series of potential antidepressants. *J Med Chem* 30: 318-325.
3. Stenger A, Couzinier J P and Briley M (1987) Psychopharmacology of milnacipran, 1-phenyl-1-diethyl-amino-carbonyl-2-aminomethylcyclopropane hydrochloride (F2207), a new potential antidepressant. *Psychopharmacology* 91: 147-153.
4. Palmier C, Puozzo C, Lenehan T and Briley M. (1989) Monoamine uptake inhibition by plasma from healthy volunteers after single oral doses of the antidepressant milnacipran. *Eur J Clin Pharmacol* 37: 235-238
5. Puech A, Montgomery S A, Prost J F, Solles A and Briley M (1997) Milnacipran, a new serotonin and noradrenaline reuptake inhibitor: an overview of its antidepressant activity and clinical tolerability. *Int Clin Psychopharmacol* 12: 99-108.
6. Christian P, Christian F and Gregoire Z (2004) Determination of milnacipran, a serotonin and noradrenaline reuptake inhibitor, in human plasma using liquid chromatography with spectrofluorimetric detection. *J Chromatogr* 806: 221-228.
7. Angela P, Sonia P and Claudia S (2007) Chiral HPLC analysis of milnacipran and its FMOc-derivative on cellulose-based stationary phases. *Chirality* 20: 63 – 68.
8. Marie Lecoœur L, Raphael D, Jean Paul R and Philippe M (2008) Chiral analysis of milnacipran by a nonchiral HPLC - circular dichroism: Improvement of the linearity of dichroic response by temperature control. *J Separ Sci* 31: 3009 – 3014.
9. The United States Pharmacopeia (2005) The National Formulary 28, US Pharmacopeial Convention Inc., Rockville, MD, USA: 2748-2751
10. ICH-Q2R1 Validation of Analytical Procedures: Methodology International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Geneva, Switzerland (1996).
11. Cazes J. Update Supplement Encyclopedia of Chromatography (2004) Validation of HPLC Instrumentation. 339
12. Armbruster D A, Tillman M D and Hubbs L M (1994) Comparison of the empirical and the statistical methods exemplified with GC-MS assays of abused drugs. *Clin Chem* 40: 1233-1238