

## VALIDATED RP-HPLC METHOD FOR ANALYSIS OF MILNACIPRAN IN BULK AND IN FORMULATIONS

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### ABSTRACT

A rapid, simple and validated reversed-phase high-performance liquid chromatographic method has been developed for analysis of Milnacipran in tablet dosage form. Milnacipran was separated on an Inertsil ODS analytical column with a 40:60 (v/v) mixture of Methanol and Octane sulfonic acid buffer (containing 0.05%w/v Octane sulfonic acid with 0.025%v/v Trifluoro acetic acid) as mobile phase at a flow rate of 1.0 mL min<sup>-1</sup>. The effluent was monitored by UV detection at 220 nm. Calibration plots were linear in the range of 10 to 50 µg mL<sup>-1</sup> and the LOD and LOQ were 0.536 and 1.624 µg mL<sup>-1</sup>, respectively. The high recovery and low relative standard deviation confirm the suitability of the method for routine quality control determination of Milnacipran in capsules.

**KEYWORDS:** Milnacipran, RP-HPLC, Capsule analysis, Validation.

### INTRODUCTION

Milnacipran, 2-(amino methyl)-*N,N*-diethyl-1-phenylcyclopropanecarboxamide (Figure 1), is a psychoactive drug. It shows an equipotent inhibitory action on serotonin and noradrenaline neuronal reuptake systems and total lack of affinity for neurotransmitter receptors, thus giving a similar efficacy to the tricyclic antidepressants in the treatment of clinical depression but with fewer side effects. The therapeutic potential of the drug could be related to its activity as *N*-methyl-D-aspartate receptor antagonist<sup>1,3</sup>. It is used for the treatment of clinical depression and fibromyalgia<sup>4-5</sup>.

Extensive literature survey revealed that the chiral determination of Milnacipran is carried out in plasma and serum using liquid chromatography (LC) coupled with UV and spectrofluorimetric detection<sup>6-8</sup> and by micellar electrokinetic capillary chromatography<sup>9</sup>. The studies had also been carried for the chiral determination of Milnacipran and its Fmoc (9-fluorenylmethoxycarbonyl) Derivative in tablet formulation on cellulose based stationary phases<sup>10</sup>. Milnacipran had also been analyzed for its enantiomeric excess and chemical purity using circular dichroism detector<sup>11</sup>. However, no simple and sensitive isocratic RP-HPLC method with UV detection is been reported for the determination of Milnacipran in pharmaceutical formulations. Hence the present research work was aimed to develop and validate<sup>12-13</sup> the simple, specific and sensitive RP-HPLC method for the determination of Milnacipran and its pharmaceutical formulation.

### MATERIALS AND METHODS

Methanol and water of HPLC grade were from Qualigens (Bombay, India) and octane sulfonic acid and trifluoro acetic acid (analytical reagent grade) were purchased from SD Fine Chemicals (Bombay, India). De-ionized water was used throughout the experiment. Before use, mobile phase was filtered through a 0.45 µm cellulose acetate filter from Millipore (USA). Whatman no. 41 filter papers (obtained commercially) were used for preparation of sample solutions.

**Chromatographic system and conditions**

Analysis was performed with a Shimadzu chromatograph comprising an LC-10 AT VP solvent-delivery module, a SPD-10A UV-visible detector, 10  $\mu$ L sample loop. Milnacipran was chromatographed on a 250 mm  $\times$  4.6 mm i.d., 5  $\mu$ m particle, Inertsil ODS-3 analytical column under reversed-phase partition conditions. The mobile phase was a 40: 60 (v/v) mixture of Methanol and Octane sulfonic acid buffer (containing 0.05%w/v Octane sulfonic acid with 0.025%v/v Trifluoro acetic acid). The flow rate was 1.0 mL min<sup>-1</sup> and the analyte was monitored at 220 nm. Before analysis the mobile phase was degassed by use of a sonicator and filtered through a 0.2  $\mu$ m injection filter. The column was equilibrated before each injection.

**Calibration**

Calibration plots were constructed by analysis of appropriate working solutions (Concentrations of 10, 20, 30, 40 and 50  $\mu$ g mL<sup>-1</sup>) of Milnacipran in the mobile phase and plotting concentration against peak-area response for each injection. Unknown samples were quantified by reference to these calibration plots.

**Sample preparation**

The contents of twenty capsules were accurately weighed. An amount of powder equivalent to 50 mg of Milnacipran was accurately weighed and transferred to a 50 mL volumetric flask. Mobile phase (25 mL) was added and the mixture was sonicated for 10 min for complete extraction of the drug and the solution was diluted to volume with mobile phase. The solution was filtered through a 0.2  $\mu$ m membrane filter. This solution was suitably diluted and injected for HPLC analysis.

**RESULTS AND DISCUSSION****Method development and optimization**

Column chemistry, solvent selectivity (solvent type), solvent strength (volume fraction of organic solvent(s) in the mobile phase), additive strength, detection wavelength and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimized, so there was no interference with the Milnacipran peak from solvent or excipients peaks. Other criteria, for example the time required for analysis, assay sensitivity, solvent noise and use of the same solvent system for extraction of the drug from formulation matrices during drug analysis, were also considered. After each change of mobile phase the column was equilibrated by passage of at least twenty column volumes of the new mobile phase. To investigate the appropriate wavelength for determination of Milnacipran, UV-visible spectra in the range 200–400 nm were acquired from a solution of the drug in the mobile phase (Systronics model 2201 spectrophotometer). From the UV spectra obtained the wavelength selected for monitoring the drug was 220 nm. Solutions of the drug in the mobile phase were injected directly for HPLC analysis and the responses (peak area) were recorded at 220 nm. It was observed there was no interference from the mobile phase or baseline disturbance at 220 nm. Therefore, it was, concluded that 220 nm was the most appropriate wavelength for analysis of the substance with suitable sensitivity.

**Chromatography**

Symmetrical peaks were obtained for Milnacipran. Typical chromatograms obtained from a blank and from a solution of the drug are illustrated in Figure 2(a&b). The retention time of Milnacipran was 4.32 min and the overall chromatographic run time was 10.0 min.

**Method Validation****Linearity**

The linearity of the method was tested using the calibration solutions described above. Plot of concentrations against responses were linear in the range of 10-50  $\mu$ g mL<sup>-1</sup> (Figure 3). The mean regression equation was  $y = 50.069x + 7.9524$ . The correlation coefficient was 0.9999. The system suitability parameters are given in Table-1

**Limits of detection and quantification**

The limit of detection (LOD) is defined as the lowest concentration of an analyte that can be readily detected but not necessarily quantified. It is usually regarded as the amount for which the signal-to-noise ratio (SNR) is 3:1. The limit of quantitation (LOQ) is defined as the lowest concentration of an analyte that can be quantified with acceptable precision and accuracy. It is usually regarded as the amount for

which the SNR is 10:1. Two types of solution, blank solution and solutions containing known, progressively decreasing concentrations of the analyte were prepared and analyzed. LOD and LOQ were 0.536 and 1.624  $\mu\text{g mL}^{-1}$ , respectively.

#### Accuracy

Recovery studies were performed in triplicate after spiking raw material in volumetric flasks with amounts of Milnacipran equivalent to 80, 100 and 120% of the standard concentration of Milnacipran (30  $\mu\text{g mL}^{-1}$ ) as in the analytical method. The results obtained (Table 2) indicate that recovery were excellent, not less than 98% and that relative standard deviations also less than 2%.

#### Precision

Intra-day precision was calculated from results obtained from six-fold replicate analysis of samples at three different concentrations on the same day. Inter-day precision was calculated from results from the same samples analyzed on three consecutive days. The results obtained are listed in Table 3.

#### Specificity

The specificity of the method was tested by chromatographing a mixture of commonly used tablet excipients, for example starch, lactose and magnesium stearate (blank placebo) and comparing the chromatogram with that obtained from a mixture of drug and the same additives (placebo). The chromatograms obtained (Figures 4a & 4b) showed separation of the analyte from the excipients was complete, i.e. there was no interference from the excipients under the chromatographic conditions used for the analysis.

#### Application of the method to capsules

The method was used for determination of Milnacipran in a capsule formulation. The results obtained (Table 4) showed the amount found was that expected and RSD (%) values were low, which confirms the method is suitable for routine analysis of the compound in pharmaceutical preparations. A typical chromatogram obtained from analysis of a capsule formulation is shown in Figure. 4b.

#### CONCLUSION

This RP-HPLC method for analysis of Milnacipran in formulations is very simple, sensitive, and accurate. The run time is 10 min only; so many samples can also be processed and analyzed in a short period of time. The procedure described is suitable for the routine estimation of Milnacipran in pharmaceutical formulations.

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**Table 1: System suitability parameters**

Parameter	Results
Retention Time (Rt) (Min)	4.32
Theoretical Plates (n)	2281
Peak asymmetry	0.91
Linearity range (µg/ml)	10 – 30
Limit of Detection (µg/ml)	0.536
Limit of Quantification (µg/ml)	1.624

**Table 2: Accuracy of the method**

Drug	Spike level, %	Concentration added, µg mL <sup>-1</sup>	Mean amount recovered, µg mL <sup>-1</sup> , (n=3)	% Recovery, (n = 3)	% RSD (n = 3)
Milnacipran Capsules	80	24	23.82	99.25	0.72
	100	30	30.24	100.8	0.54
	120	36	35.44	98.4	0.98

**Table 3: Intra-day and inter-day precision of the method**

Concentration Added, µg mL <sup>-1</sup>	Intra-day precision		Inter-day precision	
	Mean amount found, µg mL <sup>-1</sup> (n = 6)	% RSD (n = 6)	Mean amount found, µg mL <sup>-1</sup> (n = 3)	% RSD (n = 3)
24	23.62	1.04	23.84	0.95
30	30.45	0.86	30.12	0.74
36	36.14	1.22	35.89	0.8

**Table 4: Results from analysis of Milnacipran in tablets**

<b>Label claim. mg per tablet</b>	<b>50</b>
Amount found, mg per tablet	49.45
Amount found, %, n = 6	98.9
RSD, %, n = 6	0.54

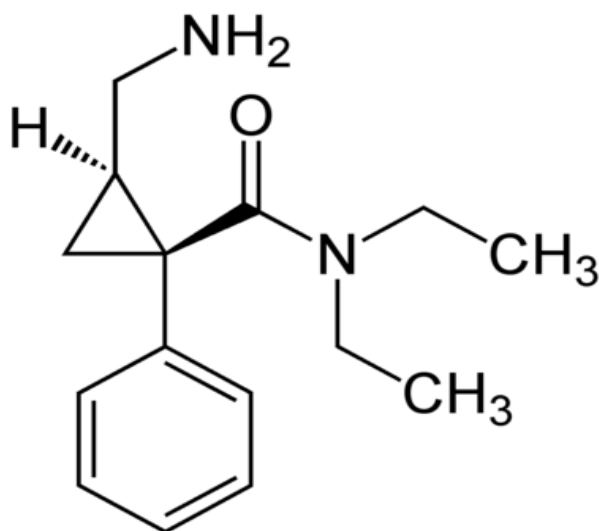


Figure 1: Structure of Milnacipran

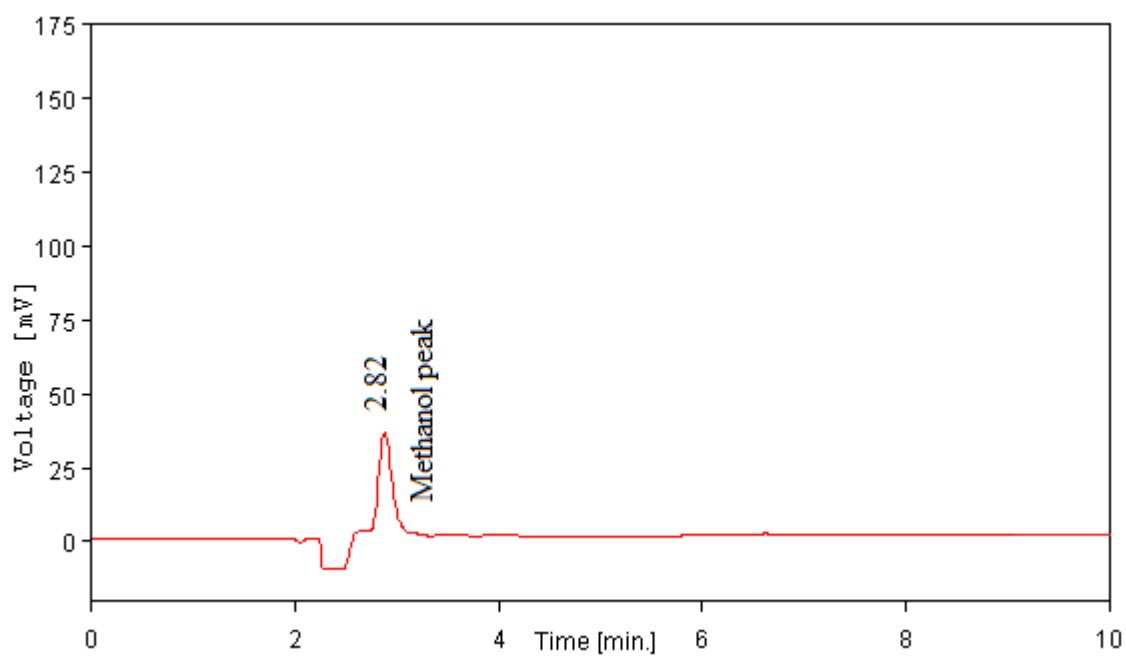


Figure 2a

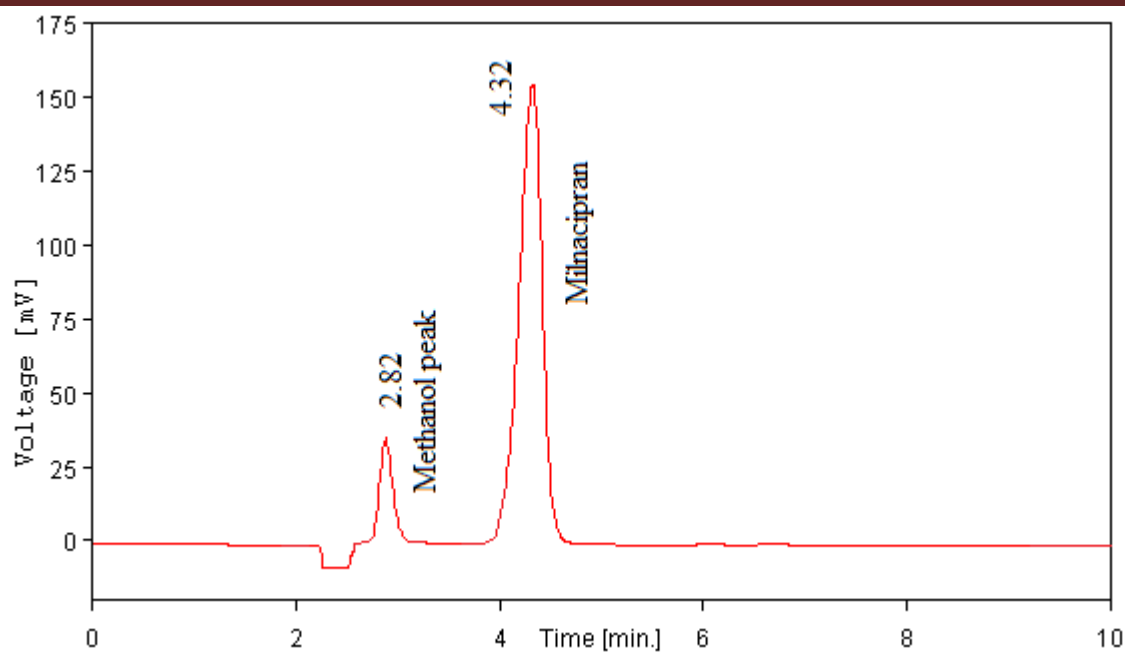


Figure 2b

Figure 2: (a) Typical chromatograms obtained from blank and (b) Milnacipran solution

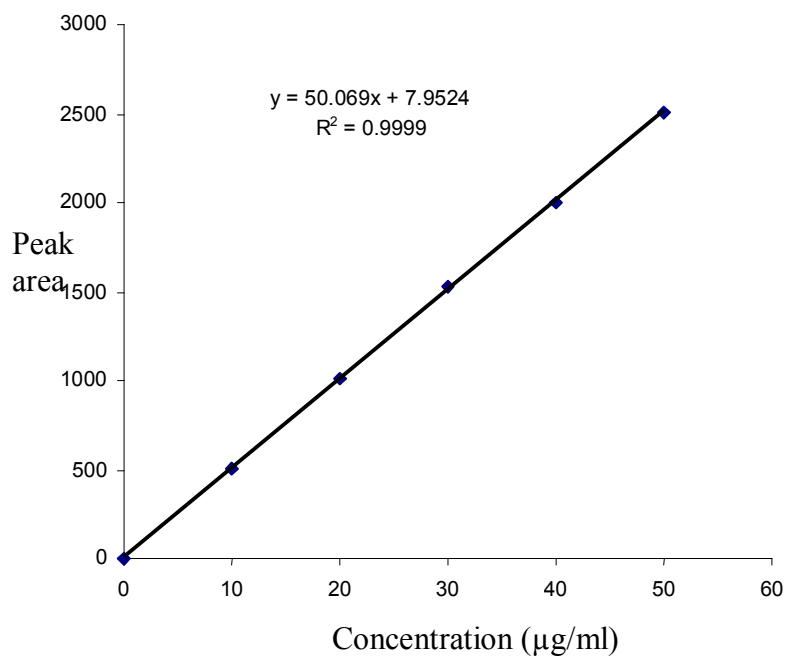


Figure 3: Calibration plot for Milnacipran

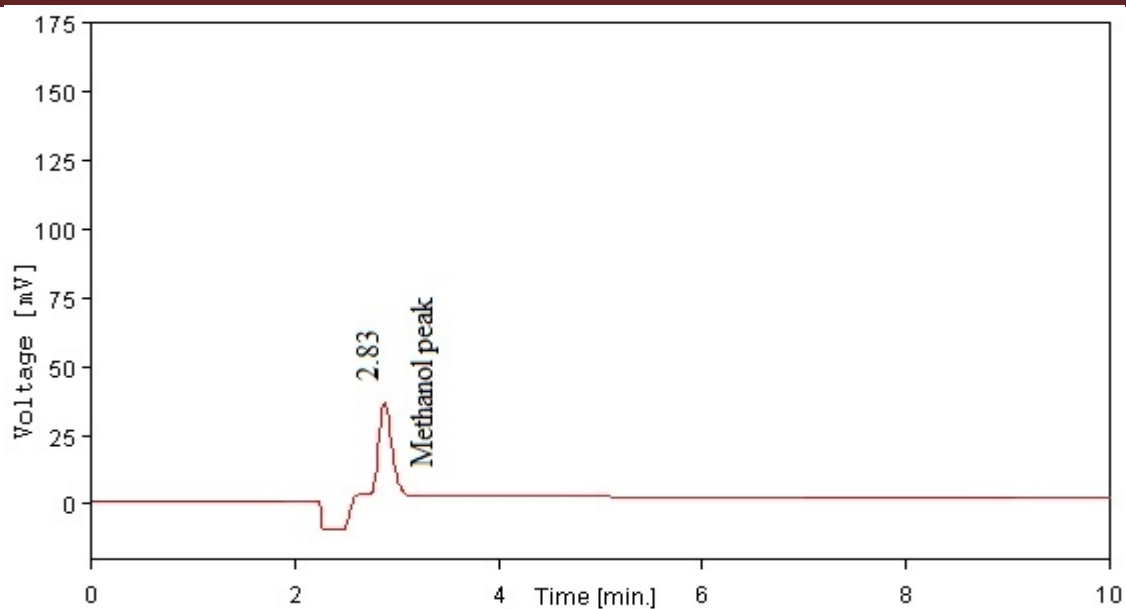


Figure 4a

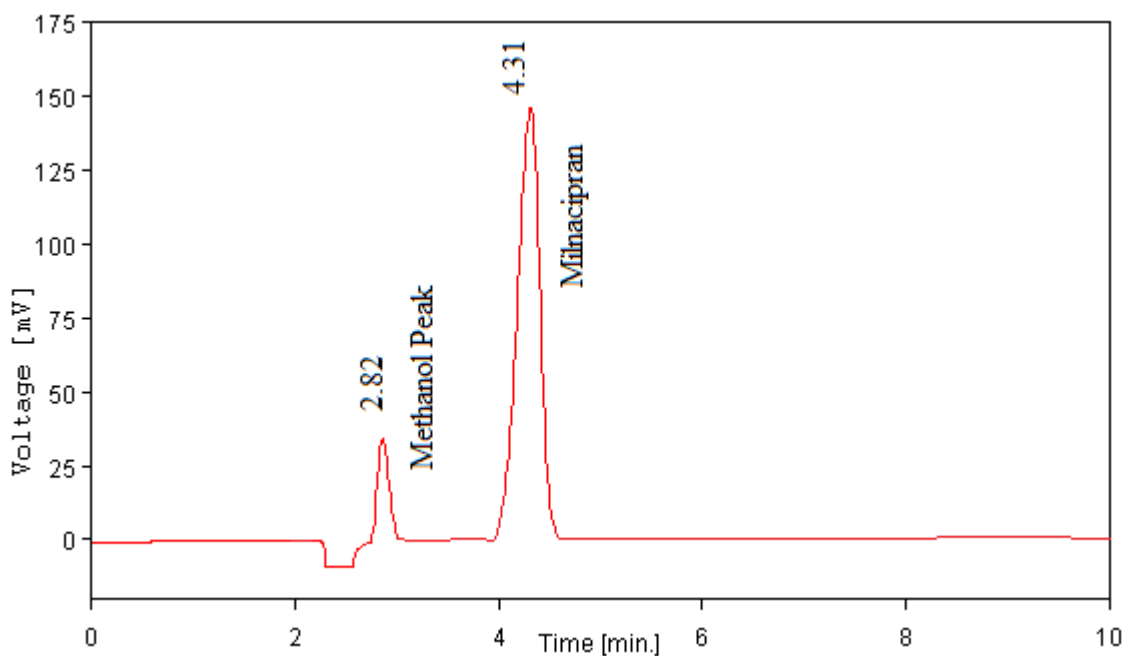


Figure 4b

Figure 4: (a) Typical chromatograms obtained from Placebo and (b) Milnacipran sample solution (capsule)

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