DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF MILNACIPRAN TABLET DOSAGE FORMS

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INTRODUCTION

Milnacipran hydrochloride is a new antidepressant agent, selective norepinephrine and serotonin reuptake inhibitor; it inhibits norepinephrine uptake with greater potency than serotonin. It is a racemic mixture. Chemically, it is (1R,2S)-rel-2-(Aminomethyl)-N,N-diethyl-1-phenylcyclopropylcarboxamide. Milnacipran inhibits norepinephrine uptake with approximately 3-fold higher potency in vitro than serotonin without directly affecting the uptake of dopamine or other neurotransmitters. Milnacipran has no significant affinity for serotonergic (5-HT1, 2a, 2c, 3, 5-HT4), α- and β-adrenergic, muscarinic (M1, M3), histamine (H1, H2), dopamine (D1, D2, D4), opiate, benzodiazepine and GABA receptors in vitro. Pharmacologic activity at these receptors is hypothesized to be associated with the various anticholinergic, sedative and cardiovascular effects seen with other psychotropic drugs. Milnacipran has no significant affinity for Ca++, K+, Na+, and Cl- channels and does not inhibit the activity of human monoamine oxidases (MAO-A and MAO-B) or acetylcholinesterase.

A few HPLC and LC-MS methods were reported earlier for the determination of milnacipran in bulk and pharmaceutical dosage forms. In the present study the authors report a rapid, sensitive, accurate and precise HPLC method for the estimation of milnacipran in bulk samples and in tablet dosage forms.

EXPERIMENTAL

Chromatographic conditions: The analysis of the drug was carried out on a Waters HPLC system equipped with a reverse phase Agilent C18 column (75mmx4.6mm; 3.5µm), a 2695 binary pump, a 20 µl injection loop and a 2487 dual absorbance detector and running on Waters Empower software.

Chemicals and solvents: The reference sample of milnacipran was supplied by Sun Pharmaceutical Industries Ltd., Baroda. HPLC grade water and acetonitrile were purchased from E. Merck (India) Ltd., Mumbai. Potassium dihydrogen phosphate and orthophosphoric acid of AR Grade were obtained from S.D. Fine Chemicals Ltd., Mumbai.

Preparation of phosphate buffer (pH 3.0): 6.8 gms of KH2PO4 was weighed into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC water. 2 ml of Triethyl amine was added.
Validation of the proposed method: The specificity, linearity, precision, accuracy, limit of detection, limit of quantitation, robustness and system suitability parameters were studied systematically to validate the proposed HPLC method for the determination of milnacipran. Solutions containing 10, 25 and 50 µg/ml of milnacipran were subjected to the proposed HPLC analysis to check intra-day and inter-day variation of the method and the results are furnished in Table 2. The accuracy of the HPLC method was assessed by analyzing solutions of milnacipran at 50, 100 and 150 % concentrated levels by the proposed method. The results are furnished in Table 3. The system suitability parameters are given in Table 4.

Estimation of milnacipran in tablet dosage forms: Two commercial brands of tablets were chosen for testing the suitability of the proposed method to estimate milnacipran in tablet formulations. Twenty tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 25 mg of milnacipran was transferred into a 100 ml volumetric flask and dissolved in 25 ml of a 75:25 v/v mixture of phosphate buffer and acetonitrile. The contents of the flask were sonicated for 15 min and a further 25 ml of the diluent was added, the flask was shaken continuously for 15 min to ensure complete solubility of the drug. The volume was made up with the diluent and the solution was filtered through a 0.45 µ membrane filter. This solution was injected into the column six times. The average peak area of the drug was computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug. The relevant results are furnished in Table 5.

Fig. 1: Chemical structure of milnacipran
RESULTS AND DISCUSSION
In the proposed method, the retention time of milnacipran was found to be 6.072 min. Quantification was linear in the concentration range of 10-100 µg/ml. The regression equation of the linearity plot of concentration of milnacipran over its peak area was found to be $Y=26585+57025X$ ($r^2=0.999$), where $X$ is the concentration of milnacipran (µg/ml) and $Y$ is the corresponding peak area. The number of theoretical plates calculated was 8237, which indicates efficient performance of the column. The limit of detection and limit of quantification were found to be 0.12 µg/ml and 0.28 µg/ml respectively, which indicate the sensitivity of the method. The use of phosphate buffer and acetonitrile in the ratio of 75:25 v/v resulted in a peak with good shape and resolution. The high percentage of recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in tablet formulations did not interfere with the estimation of the drug by the proposed HPLC method.

CONCLUSION
The proposed HPLC method is rapid, sensitive, precise and accurate for the determination of milnacipran and can be reliably adopted for routine quality control analysis of milnacipran in its tablet dosage forms.
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REFERENCES