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Research Article

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

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ABSTRACT

An accurate and precise HPLC method was developed for the determination of milnacipran. Separation of the drug was achieved on a reverse phase C_{18} column using a mobile phase consisting of phosphate buffer and acetonitrile in the ratio of 75:25 v/v. The flow rate was 1.0 ml/min and the detection wavelength was 220 nm. The linearity was observed in the range of 10-100 µg/ml with a correlation coefficient of 0.999. The proposed method was validated for its linearity, accuracy, precision and robustness. This method can be employed for routine quality control analysis of milnacipran in tablet dosage forms.

Keywords: Milnacipran, Estimation, RP-HPLC, Validation.

INTRODUCTION

Milnacipran hydrochloride[1] 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[2], it is (1*R*,2*S*)-*rel*-2-(Aminomethyl)-*N*,*N*-diethyl-1-phenylcyclo propanecarbox-amide. 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-HT_{1-7}), α- and β-adrenergic, muscarinic (M_{1-5}), histamine (H_{1-4}), dopamine (D_{1-5}), opiate, benzodiazepine and □-aminobutyric acid (GABA) receptors *in vitro*. Pharmacologic activity[3] 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[4] and LC-MS[5-7] 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 C_{18} 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 KH_2PO_4 was weighed into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC water. 2 ml of Triethyl amine was added

and pH adjusted to 3.0 with orthophosporic acid.

Preparation of mobile phase and diluent: 750 ml of the phosphate buffer was mixed with 250 ml of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µ filter under vacuum.

Procedure: A mixture of buffer and acetonitrile in the ratio of 75:25 v/v was found to be the most suitable mobile phase for ideal separation of milnacipran. The solvent mixture was filtered through a 0.45 µ membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1.0 ml/min. The column was maintained at ambient temperature. The pump pressure was set at 1250 psi. The column was equilibrated by pumping the mobile phase through the column for at least 30 min prior to the injection of the drug solution. The detection of the drug was monitored at 220 nm. The run time was set at 8 min. Under these optimized chromatographic conditions the retention time obtained for the drug was 6.072 min. A typical chromatogram showing the separation of the drug is given in Fig. 2.

Calibration plot: About 25 mg of milnacipran was weighed accurately, 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 solution was sonicated for 15 min and the volume made up to the mark with a further quantity of the diluent to get a 250 µg/ml solution. From this, a working standard solution of the drug (25 µg/ml) was prepared by diluting 1 ml of the above solution to 10 ml in a volumetric flask. Further dilutions ranging from 10-100 µg/ml were prepared from the solution in 10 ml volumetric flasks using the above diluent. 20 µl of each dilution was injected six times into the column at a flow rate of 1.0 ml/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed. The calibration graph constructed by plotting concentration of the drug against peak area was found to be linear in the concentration range of 10-100 µg/ml of the drug. The relevant data are furnished in Table 1. The regression equation of this curve was computed. This regression equation was later used to estimate the amount of milnacipran in tablet dosage forms.

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.

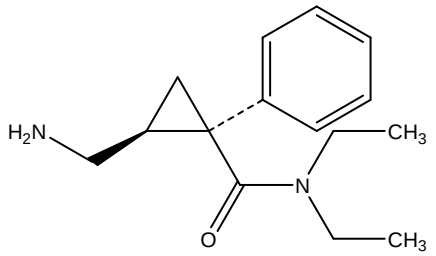

Fig. 1: Chemical structure of milnacipran

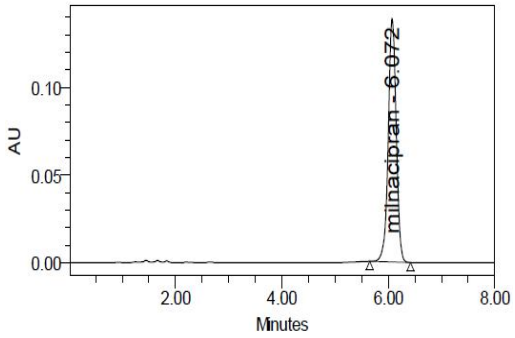

Fig. 2: Typical chromatogram of milnacipran

Table 1: Calibration data of the method

Concentration (µg/ml)	Mean peak area (n=6)
10	595842
25	1423891
50	2915351
75	4316963
100	5707381

Table 2: Precision data of the proposed HPLC method

Concentration of milnacipran (µg/ml)	Measured concentration of milnacipran (µg/ml)			
	Intra-day		Inter-day	
	Mean (n=3)	% C.V.	Mean (n=3)	% C.V.
10	9.97	0.124	9.95	0.111
25	25.09	0.524	24.31	0.091
50	50.61	0.234	50.37	0.030

Table 3: Accuracy studies

Concentration	Amount added (mg)	Amount found (mg)	% Recovery	% Mean recovery
50%	12.5	12.4	99.2	99.4
100%	24.9	24.8	99.8	
150%	37.6	37.3	99.2	

Table 4: System suitability parameters

Parameter	Result
Linearity ((µg/ml)	10-100
Correlation coefficient	0.999
Theoretical plates (N)	8237
Tailing factor	0.98
LOD (µg/ml)	0.12
LOQ (µg/ml)	0.28

Table 5: Assay and recovery studies

Formulation	Label claim (mg)	Amount found (mg)	% Amount found
Milnace	25	24.9	99.8
Acmil	25	24.8	99.0

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 8238, 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 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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