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Research Article

A NEW VALIDATED RP – HPLC METHOD FOR DETERMINATION OF MILNACIPRAN IN TABLET DOSAGE FORMS

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Abstract: A simple, sensitive and precise reverse phase high performance liquid chromatographic method has been developed for the estimation of Milnacipran in pharmaceutical dosage forms. The mobile phase consist of buffer (0.02M sodium dihydrogen phosphate, pH-3.6 adjusted with ortho phosphoric acid): acetonitrile in the ratio of 60:40 v/v delivered at a flow rate of 1.0 ml / min and wavelength of detection at 220 nm. The retention time of Milnacipran was 4.22 min. The developed method was validated according to ICH guidelines. The result indicates that the method was found to be simple, rapid, and accurate and can be adopted in routine analysis of Milnacipran in Pharmaceutical dosage forms.

Key**words:** Milnacipran, RP-HPLC, Validation, ICH

INTRODUCTION

Milnacipran HCL is used in the treatment of fibromyalgia and depression[1-2]. Its chemical name is 1-diethyl amino methyl -1-phenyl-cyclopropane HCl, it belongs to the family 1-aryl-2-amino methyl cyclopropane carboxylic acid derivatives. It potentially inhibits the reuptake action of both neurotransmitters serotonin and epinephrine.[1]

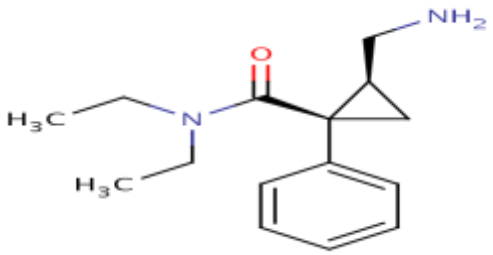

Fig-1: Chemical structure of Milnacipran

It has been reported that Milnacipran is estimated by Simple Chromotography[3],RP-HPLC[4-6], LC/MS [7] , U-HPLC[8], HPTLC[9] and UV-Spectrophotometer[10] . The present work tends to design a simple RP-HPLC method using C18 column for determination of Milnacipran in tablet combined dosage form. The method was validated as per ICH guidelines. [11]

EXPERIMENTAL SECTION:

Reagents and Chemicals:

Milnacipran API was obtained as gift sample from HETERO laboratories limited, Hyderabad. The branded formulations (tablets) (Savella tablets containing 50mg of Milnacipran) were procured from the local market. Acetonitrile, Methanol, Sodium dihydrogen phosphate, Water and ortho phosphoric acid used were of HPLC grade and purchased from Merck Specialities Private Limited, Mumbai, India.

Instrumentation:

Chromatographic separation was performed on a Shimadzu chromatographic system (class VP series) equipped with two LC-10AT VP pumps; variable wavelength programmable UV/VIS detector, SPD-20A and Rheodyne injector (7725i) with 20µl fixed loop. Chromatographic conditions: Enable C18 (150 x 4.6mm, 5µ) was the column used for separation. Mobile phase consisting of a mixture of Buffer (0.02M sodium dihydrogen phosphate, pH-3.6 was adjusted with ortho phosphoric acid), and acetonitrile in the ratio 60:40 v/v was delivered at a flow rate of 1.0 ml/min with detection at 220 nm. The mobile phase was filtered through a 0.45 nylon filter and sonicated for 15 min. Analysis was performed at ambient temperature. Pharmaceutical dosage form: Commercial tablets (Savella) were procured randomly from the local market.

Method development:

Buffer (0.02M sodium dihydrogen phosphate pH-3.6 was adjusted with ortho phosphoric acid) and Acetonitrile in different proportions were tried and finally Buffer (0.02M sodium dihydrogen phosphate, pH-3.6 was adjusted with ortho phosphoric acid) and acetonitrile (60: 40 v/v) was selected as an appropriate mobile phase which gave good resolution and acceptable system suitability parameters. The chromatogram of working standard solution is shown in Fig-2.

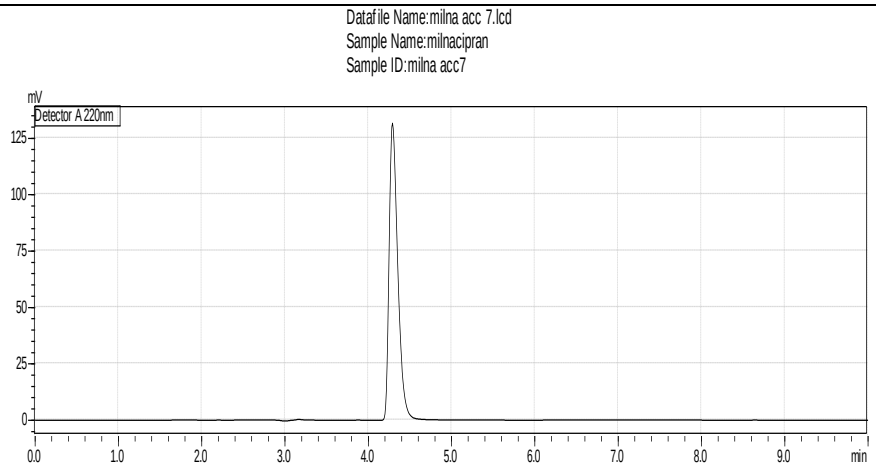

Fig-2: The chromatogram of working standard Milanacipran

PROCEDURE:

Preparation of standard solution: Weigh and transfer accurately about 50.0 mg of Milnacipran standard into a 100 ml clean dry volumetric flask, add about 60 ml of diluent, sonicate for 5 minutes, and dilute to volume with diluent. Transfer 5ml of this of this solution into a 50ml volumetric flask and dilute to the volume with diluent. Filter the solution through 0.45µ nylon membrane filter, inject 10µl of the solution into chromatograph and record the chromatogram.

Procedure for analysis of tablets:

Weigh and powder not less than twenty tablets. Accurately weigh and transfer tablet powder equivalent to 50.0mg of Milnacipran into 100ml volumetric flask, add about 60ml of diluents and sonicate for 5 mins. Make up the volume with diluent. Transfer 5.0 ml of the solution to 50 ml of volumetric flask and dilute to the volume with diluent. Filter the solution through 0.45µm nylon filter with the optimized chromatographic conditions, a steady baseline was recorded, the working standard solution was injected into the chromatograph and the chromatogram was recorded. The retention time of Milnacipran was found to be 4.22 mins. The proposed method was found to be specific and no interference from common tablet excipients was observed. The response factors of the standard solutions and sample solutions were calculated. The assay was calculated from the equation of regression line for each drug. The assay procedure was repeated for 6 times and the percentage of individual drug in the formulation was calculated. The results of analysis shows that the amount of drug was in good agreement with the label claim of formulation (Table-1).

Table-1: Analysis of tablet formulation

Formulation	Analyte	Label claim (mg)	%Label claim estimated
Tablet	Milnacipran	50	98.9

RESULT

Calibration curve:

Accurately measured volume of working standard solution of Milnacipran was transferred into a series of 10ml volumetric flasks and diluted appropriately with mobile phase. 20µl of each solution was injected under operating chromatographic conditions described above. Calibration curves were obtained by plotting the response (area of drug peak) versus concentration of drug. Regression equations were calculated. The method was found linear over a concentration range of 5µg/ml to 25µg/ml

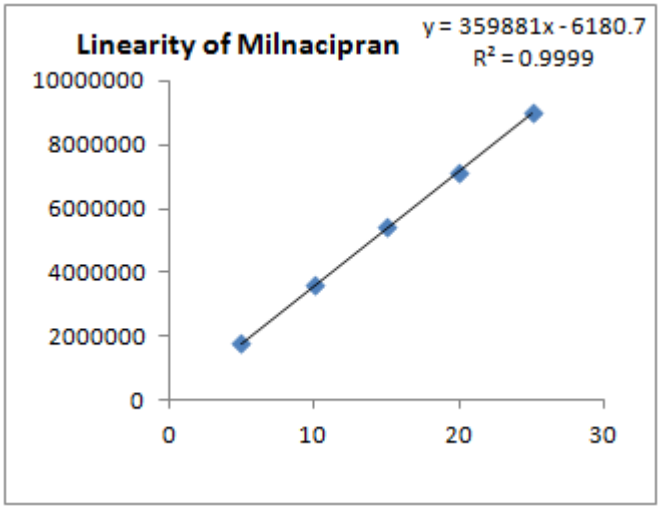

Fig-3: Calibration curves of Milanacipran

METHOD VALIDATION

System suitability:

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions. The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 % standard deviation range during routine performance of the method.

Accuracy:

The accuracy of the method was determined by recovery experiments. Known concentration of working standard was added to the fixed concentration of the pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of working standard. For both the drugs, recovery was performed in the same way. The recovery studies were performed in triplicate. This standard addition method was performed at 50%, 100%, 150% level and the percentage recovery were calculated. Percent recovery was within the range of 98.59

to 100.09 for Milnacipran which indicates that the method was accurate. The Accuracy data was given in Table 2.

Table 2: Accuracy or Recovery of Milnacipran

Concentration of % spiked level	Amount recovered	% Recovery
50% Sample 1	50.05	100.09
50% Sample 2		
50% Sample 3		
100% Sample 1	99.48	99.48
100% Sample 2		
100% Sample 3		
150% Sample 1	147.89	98.59
150% Sample 2		
150% Sample 3		

Precision:

The precision of the method was demonstrated by inter day and intraday variation studies. In the intraday studies, solutions of standard and sample were repeated thrice in a day and percent relative standard deviation (%RSD) for response factor was calculated. The intraday %RSD of Milnacipran was found to be 0.04. In the interday variation studies, injections of standard and sample solutions were made on three consecutive days and %RSD was calculated. The interday %RSD for Milnacipran was found to be 0.05. From the data obtained the developed RP-HPLC method was found to be precise.

Linearity:

The method was linear in the range of 5 µg/ml to 25 µg/ml for Milnacipran. Linear regression data was given in Table 3.

Table 3: Linear regression data for calibration curves

Parameter	Milnacipran
Linearity range (µg/ml)	5-25
Correlation coefficient	0.999

Robustness:

Robustness of the method was checked by making slight deliberate changes in chromatographic conditions like mobile phase composition, pH of buffer, flow rate and temperature variation. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed RP-HPLC method is robust. The result was shown in Table 4.

Table 4: Robustness for Milnacipran

S No	Sample name	Change	Name	RT	Area	Tailing	Plate count
1	Flow 1	1.1 ml/min	Milnacipran	4.23	5445236	1.03	3029
2	Flow 2	0.9 ml/min	Milnacipran	4.19	5208757	1.10	3454

Effect of variation in mobile phase composition:

A study was conducted to determine the effect of variation in organic phase composition in mobile phase. Standard solution prepared as per the test method was injected into the HPLC system using two mobile phases. The system suitability parameters were evaluated and found to be within the limits for mobile phase having 90% and 110% of method highest organic phase. Milnacipran blend solution at target concentration was chromatographed using mobile phase having 90% and 110% of the method organic phase. Milnacipran was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having 100% of the organic phase. From the study it was established that the allowable variation in mobile phase composition is 90% to 110% of the method highest organic phase of mobile phase.

Effect of variation of flow rate:

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 0.9ml/min and 1.1ml/min. The system suitability parameters were evaluated and found to be within the limits for 0.9ml/min and 1.1ml/min flow. Milnacipran was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having flow rates 1.0ml/min. From the above study it was established that the allowable variation in flow rates is 0.9ml/min and 1.1ml/min.

Effect of variation of pH:

A study was conducted to determine the effect of variation in pH Standard and sample solutions were prepared as per the test method and injected into the HPLC system using pH 3.4 and 3.8. The system suitability parameters were evaluated and found to be within the limits for pH 3.4 and 3.8. Milnacipran were resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having pH 3.6.From the above study it was established that the allowable variation in pH 3.4 and 3.8 Hence the method is robust.

Ruggedness:

Ruggedness is performed by the analysis of same batch will be done in six analysis by using columns of the same make having different serial numbers by different analysts, by different systems. The mean standard deviation and %RSD is calculated. The %RSD was found to be 0.05.

Table 5: Ruggedness of Milnacipran

Milnacipran	Retention time	Area
Sample 1	4.220	5641458
Sample 2	4.223	5598756
Sample 3	4.221	5680478
Sample 4	4.225	5598997
Sample 5	4.219	5654571
Sample 6	4.221	5687655
Average	4.2215	5643653
Std dev	0.002168	38538.75
%RSD	**0.05**	**0.68**

DISCUSSION

The proposed method was found to be linear in the concentration range of 5 to 25 µg/ml for Milnacipran. The method was specific since excipients in the formulation did not interfere in the estimation of Milnacipran. Accuracy of the method was indicated by recovery values from 98.59 to 100.09 % for Milnacipran. Precision is reflected by %RSD values less than 2. These low values suggest sensitivity of the developed method. Validation parameters were summarized in Table 6.

Table 6: Summary of validation parameters

Parameter	Saxagliptin
Accuracy (mean % Recovery)	98.59 to 100.09
Precision	
a)System precision	0.05
b)Method precision	0.04
Retention time	4.22
Linearity	5-25
Robustness	Robust

CONCLUSION

The developed RP-HPLC method was simple, sensitive, precise and accurate and hence can be used in routine for the determination of Milnacipran in bulk as well as in pharmaceutical preparations.

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