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Research Article

STABILITY INDICATING RP-HPLC METHOD FOR THE ESTIMATION OF MILNACIPRAN HYDROCHLORIDE IN BULK AND ITS PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: To develop and validate a stability indicating reverse phase high performance liquid chromatographic method for the determination of Milnacipran hydrochloride in bulk and its pharmaceutical dosage form.
Methods: The estimation was carried out on Agilent 1200 HPLC model equipped with UV detector using Kromasil C_{18} (100 × 4.6 mm, 5 µ particle size) column with potassium phosphate buffer (pH 3.3) and methanol (55:45, V/V) as mobile phase at a flow rate of 1.0 ml/min with UV detection at 220 nm.
Results: The retention time was found to be 3.56 min and the proposed method was linear in the concentration range of 25-150 µg/ml (r^2 = 0.999) with regression equation y = 25917x - 19637. The forced degradation studies were performed by using hydrochloric acid (0.1 N HCl), sodium hydroxide (0.1 N NaOH), hydrogen peroxide (10 % H_2O_2), thermal and UV radiation. The results shown that Milnacipran hydrochloride was resistant to acidic, alkaline, oxidative, thermal and UV degradation.
Conclusion: The developed method was validated as per ICH guidelines and can successfully be applied for the routine estimation of Milnacipran hydrochloride in bulk and formulations.

Keywords: HPLC, Milnacipran hydrochloride, ICH, Kromasil C_{18}

INTRODUCTION

Milnacipran is the first in a new class of serotonin-nor epinephrine reuptake inhibitor. Milnacipran is an anti depressant and also used in the clinical treatment of fibromyalgia [1-3]. Milnacipran inhibits nor epinephrine uptake with approximately three folds higher potency *in vitro* than serotonin without directly affecting the uptake of dopamine or other neurotransmitters [4-6]. Chemically it is [2-(aminomethyl)-*N*,*N*-diethyl-1-phenylcyclopropane carboxamide] hydrochloride as shown in Figure1. with empirical formula $C_{15}H_{23}ClN_2O$ and molecular weight 282.81.

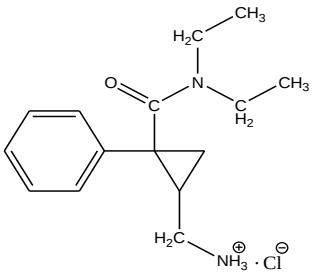

Fig. 1: Structure of Milnacipran hydrochloride

There are only few methods are available for the estimation of Milnacipran hydrochloride in pharmaceutical dosage forms using HPLC [7-11], LC/MS [12], GC-MS [13] and Spectrophotometry [14], but there is no stability indicating method using RP-HPLC for analyzing milnacipran hydrochloride in bulk and its pharmaceutical dosage forms.

MATERIALS AND METHODS

Chemicals and instrumentation

Milnacipran hydrochloride (purity 99.80 %) was obtained as a gift sample from Perkin pharmaceuticals, India. All the chemicals and reagents were obtained from Merck, Mumbai, India and were of analytical grade and HPLC grade water was purchased from Qualigens, India. Milnacipran is available as capsules (label claim: 50 mg) with brand name MILBORN manufactured by Sun pharmaceuticals, India.

Chromatographic separation was achieved on C_{18} Kromasil (100 x 4.6 mm, 5 µ particle size) column using Agilent 1200 HPLC equipped with UV detector, maintained at 30 °C column temperature. Isocratic elution was performed using potassium dihydrogen phosphate buffer (pH 3.3) and methanol (55:45, V/V). All solutions were filtered through a 0.45 µm membrane filter prior to injection.

Preparation of standard and linearity solutions of Milnacipran hydrochloride

50 mg of Milnacipran hydrochloride was accurately weighed and transferred in to a 50 ml volumetric flask. It was then dissolved in 30 ml of mobile phase and then made up to the volume with mobile phase and sonicated for 5 min (Stock solution). From this, a working standard solution of 100 µg/ml of strength was prepared. From the above stock solution by taking suitable aliquots 25 µg/ml, 50 µg/ml, 75 µg/ml, 100 µg/ml, 125 µg/ml & 150 µg/ml linearity solutions were prepared.

Preparation of sample solution

Twenty tablets were weighed and finely powdered and an accurately weighed portion of the powder equivalent to 50 mg of Milnacipran hydrochloride was transferred in to 50 ml volumetric flask. 30 ml of mobile phase was added and sonicated for 5 min with intermittent shaking and the volume is then made up to the mark with mobile phase. The resulting solution was thoroughly mixed and filtered through a 0.45 µm membrane filter prior to injection. From this, a solution of 100 µg/ml of strength was prepared.

Forced degradation studies

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method. All solutions used in forced degradation studies were prepared at initial concentrations of 1 mg/ml of Milnacipran hydrochloride and refluxed for 30 min at 80 °C. All the samples were then diluted with

diluent to give a final concentration of 100 µg/ml and filtered through a 0.45 µm membrane filter before injection.

Acid and alkali degradation studies

Acid degradation was carried out in 0.1 N HCl and refluxed for 30 min at 80 °C. After cooling, the solutions were neutralized and diluted with diluent.

Similarly, alkaline degradation was carried out in 0.1 N NaOH and refluxed for 30 min at 80 °C. After cooling, the solutions were neutralized and diluted with diluent.

Oxidation

Solutions for oxidative stress studies were prepared using 10 % H_2O_2 at a concentration of 1 mg/ml of Milnacipran hydrochloride. After refluxation for 30 min at 80 °C on the thermostat the sample solution was cooled and diluted accordingly with diluent.

Thermal degradation study

For thermal stress testing, the drug solution (1 mg/ml) was heated in thermostat at 80 °C for 30 min, cooled and analyzed.

Photo stability

The drug solution (1 mg/ml) for photo stability testing was exposed to UV light for 6 h in a UV light chamber (365 nm) and analyzed.

Method validation

The method was validated using parameters like system suitability, linearity, precision, accuracy and robustness [15-17].

System suitability

Standard solution was injected six times into system and chromatograms were recorded as shown in Figure 2. % RSD (% Relative Standard Deviation) of retention time, peak area, theoretical plates and tailing factor were calculated.

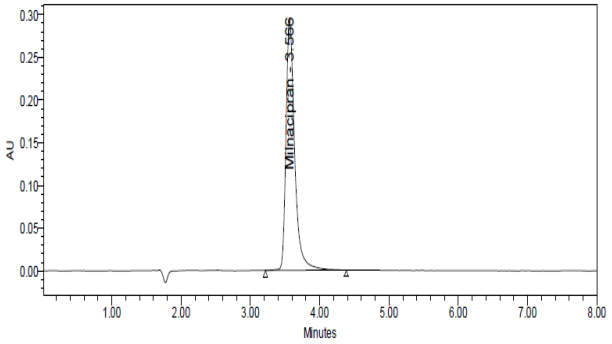

Fig. 2: System suitability peak of Milnacipran hydrochloride

Linearity

Linearity test solutions for the assay method were prepared from a stock solution at different concentration levels of 25, 50, 75, 100, 125 and 150 µg/ml and 10µl of each solution was injected into the HPLC system and the peak area of the chromatogram obtained was recorded and graph was plotted between concentration Vs peak area, r^2 was noted.

Precision

The method precision, system precision and intermediate precision were carried out using sample and standard concentration of 100 µg/ml of Milnacipran hydrochloride.

Accuracy

The accuracy of the assay method was evaluated by preparing sample solutions in triplicate at three concentration levels (50, 100 and 150 %), and predetermined amount of standard was added to these solutions. % recovery was then calculated by assaying the solutions.

Robustness

The robustness of the assay method was established by introducing small changes in the optimized HPLC conditions which included percentage of methanol (50 and 40 %), temperature (25 and 35 °C), and change in flow rate (0.8 and 1.2 ml/min). Robustness of the method was studied using six replicates at a concentration level of 100 µg/ml of Milnacipran hydrochloride.

Specificity

Standard solution, sample solution, blank solution and placebo solution were injected simultaneously into the system and chromatograms were recorded.

RESULTS AND DISCUSSION

Linearity was evaluated in the concentration range of 25-150 µg/ml. The calibration curve (as shown in Figure 3 was described by the equation, y = 25917x – 19637 with correlation coefficient 0.999. The % RSD in precision, accuracy and robustness studies were found to be less than 2.0 %, indicating that the method was precise, accurate and robust. Data of recovery study was shown in Table 1.

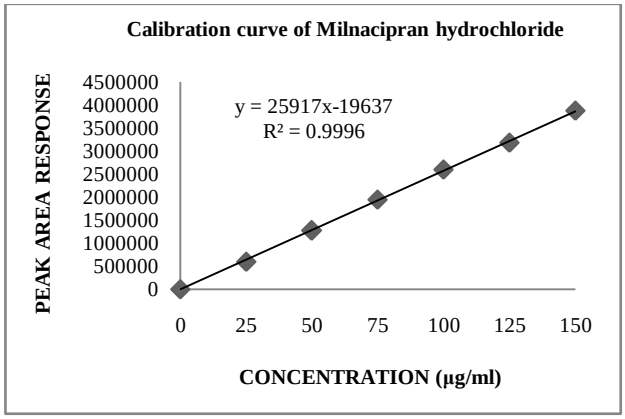

Fig. 3: Calibration curve of Milnacipran hydrochloride

Table 1: Recovery study of Milnacipran hydrochloride

S. No	Accuracy	Peak area	Amount added (µg/ml)	Amount recovered (µg/ml)	% recovery	Mean % recovery
1.	50 %	1266678	50	49.5	99.03	Mean = 98.92
2.	50 %	1267946	50	49.6	99.13	SD = 0.279
3.	50 %	1251224	50	49.3	98.60	% RSD = 0.28
4.	100 %	2545113	100	99.5	99.49	Mean = 99.44
5.	100 %	2541737	100	99.4	99.36	SD = 0.07
6.	100 %	2544648	100	99.5	99.47	% RSD = 0.07
7.	150 %	3904218	150	152.6	101.75	Mean = 101.25
8.	150 %	3886468	150	151.9	101.28	SD = 0.52
9.	150 %	3864403	150	151.1	100.71	% RSD = 0.51

The developed method is a stability indicating RP-HPLC which was not reported earlier and also specific because the drug peak was well separated even in the presence of degradation products. The representative chromatogram obtained for the drug is shown in Figure 4A, 4B, 4C, 4D and 4E. The data of forced degradation studies of Milnacipran hydrochloride was shown in Table 2.

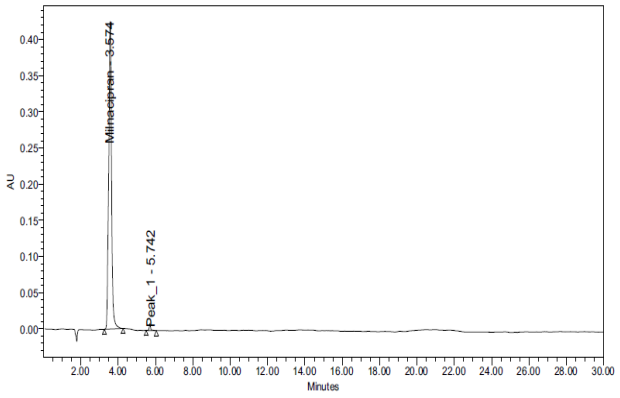

Fig. 4A: Chromatogram of Milnacipran hydrochloride on acid hydrolysis

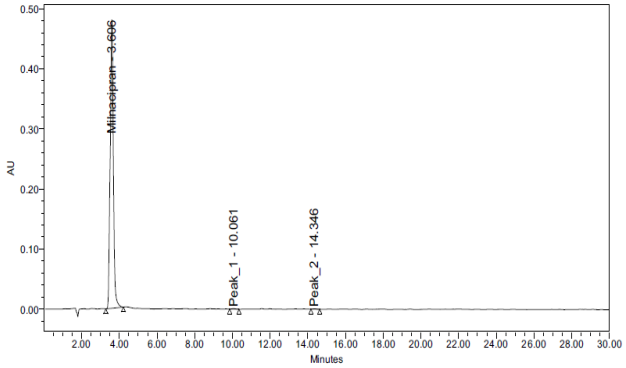

Fig. 4B: Chromatogram of Milnacipran hydrochloride on base hydrolysis

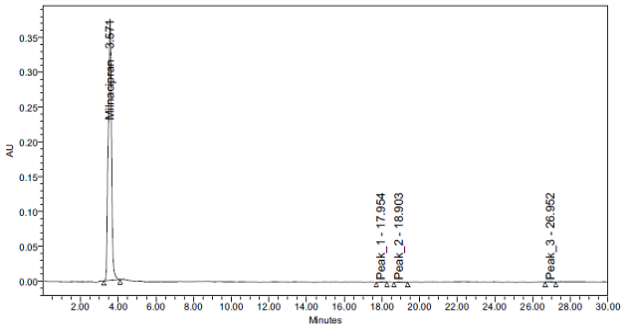

Fig. 4C: Chromatogram of Milnacipran hydrochloride on peroxide oxidation

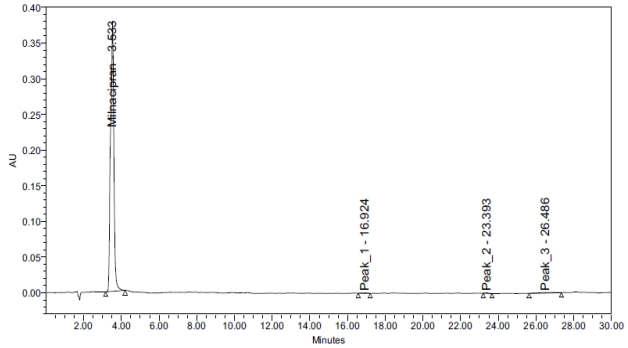

Fig. 4D: Chromatogram of Milnacipran hydrochloride on thermal degradation

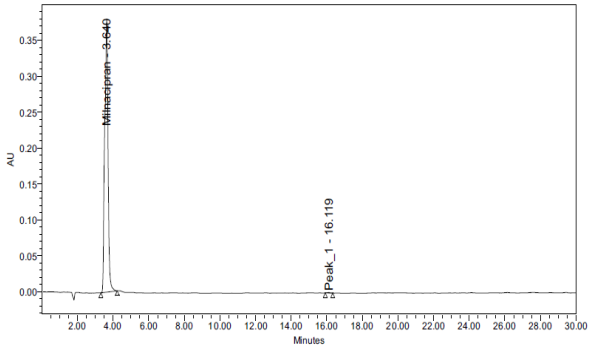

Fig. 4E: Chromatogram of Milnacipran hydrochloride on photolytic degradation

Table 2: Forced degradation studies of Milnacipran hydrochloride

Stress conditions	Drug recovered (%)	Drug decomposed (%)
Standard drug	100	-
Acid hydrolysis	99.59	0.41
Alkaline hydrolysis	98.18	1.82
Oxidative degradation	97.12	2.88
Thermal degradation	97.05	2.95
Photolytic degradation	98.91	1.09

System suitability parameters were shown in Table 3. The assay results obtained from the marketed formulations were given in Table 4.

Table 3: System suitability parameters of Milnacipran hydrochloride

S. No	Name	Retention time	Area	USP plate count	USP tailing
1	Milnacipran hydrochloride	3.566	2541737	4348	1.43

Table 4: Assay of Milnacipran hydrochloride in commercial formulation

Formulation	Label claim	Amount found	% Assay
MILBORN	50mg	49.16	98.32

Milnacipran hydrochloride shows resistant to acidic, alkaline, oxidative, thermal and photolytic degradation. It shows more degradation in thermal degradation. At thermal degradation it shows extra peaks at 16.924, 23.393 and 26.486 min. The summary of the validation parameters are presented in Table 5.

Table 5: Summary of method validation parameters

S.No	Parameter	Results
1.	System suitability	Tailing factor for Milnacipran peak is 1.43.
2.	Assay	% Assay of Milnacipran is found to be 98.32.
3.	Accuracy	Mean % recovery is 99.87.
4.	System precision	% RSD for peak areas of Milnacipran is 0.5.
5.	Method precision	% RSD for peak areas of Milnacipran is 0.82.
6.	Linearity	The correlation coefficient value is 0.999.

Effect of flow rate variation (± 0.2 ml/min)

Flow rate	R_t (min)	Tailing factor	USP plate count
0.8 ml/min	3.903	1.47	4641
0.8 ml/min	3.895	1.48	4698
1.2 ml/min	3.183	1.43	4197
1.2 ml/min	3.190	1.43	4329

		Effect of mobile phase variation (± 10 % organic)			
		Mobile phase (V/V)	R_t (min)	Tailing factor	USP plate count
7.	Robustness	60 : 40	2.091	1.43	4588
		60 : 40	2.093	1.47	4690
		50 : 50	2.136	1.50	4819
		50 : 50	2.140	1.42	4820
		Effect of temperature variation (± 5 °C)			
		Temperature (°C)	R_t (min)	Tailing factor	USP plate count
		25	3.516	1.45	4395
		25	3.501	1.45	4337
		35	3.507	1.46	4383
		35	3.500	1.46	4523

CONCLUSION

The developed and validated stability-indicating RP-HPLC method was found to be precise, accurate and robust, can be applied for the determination of Milnacipran hydrochloride in bulk and its pharmaceutical dosage forms. No interference from any components of marketed formulation or degradation products was observed and the method has been successfully used to perform long-term and accelerate stability studies of Milnacipran hydrochloride.

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