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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF MILNACIPRAN HYDROCHLORIDE IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A sensitive, isocratic RP-HPLC method was developed for the determination of Milnacipran hydrochloride in bulk drug and its pharmaceutical capsule formulations where the mobile phase optimized was phosphate buffer: acetonitrile (72:28 v/v) with C18 column as stationary phase. The flow rate and detection wavelength was 1.0 mL min^{-1} and 220 nm respectively. The developed method was validated as per ICH guidelines for specificity, linearity and range, precision, accuracy, robustness, solution stability, limit of quantification, and limit of detection and. The results of all the validation parameters were well within their acceptance values also the degradation products formed during the different stress conditions in specificity studies were separated from Milnacipran hydrochloride and also from individual degradation products. The method gave good recovery in the range of 99.1-101.0 % for Milnacipran hydrochloride when it was applied for its determination in pharmaceutical capsule formulations.

Keywords: Milnacipran hydrochloride, RP-HPLC, Validation, Pharmaceutical formulation

INTRODUCTION

Milnacipran hydrochloride, (MIL) ((Z)-1-diethylaminocarbonyl-2-aminoethyle-1-phenyl-cyclopropane hydrochloride), (Figure 1), is an antidepressant drug, belonging to the class of selective serotonin reuptake inhibitors. 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 [1-3].

Extensive literature survey revealed that the achiral determination of MIL is carried out in plasma and serum using liquid chromatography (LC) coupled with UV and spectrofluorometric detection [4-6] and by micellar electrokinetic capillary chromatography [7]. The studies had also been carried for the chiral determination of MIL and its FMOC (9-fluorenyl-methoxycarbonyl) derivative in tablet formulation on cellulose based stationary phases [8]. MIL had also been analyzed for its enantiomeric excess and chemical purity using circular dichroism detector[9]. However, no simple and sensitive isocratic RP-HPLC method with UV detection is been reported for the determination of MIL in pharmaceutical formulations. Hence the present research work was aimed to develop and validate the simple, specific and sensitive RP-HPLC method for the determination of MIL and its pharmaceutical formulation.

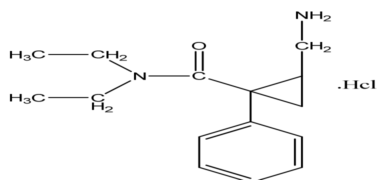

Fig. 1 Chemical structure of MIL

Experimental

Chemicals and Materials

Analytically pure MIL (purity 99.9 %) was obtained as gift sample from M/s Torrent Pharmaceutical Ltd., (Ahmedabad, India). Methanol, acetonitrile, water (Merck, Mumbai, India) were of HPLC grade, while potassium dihydrogen phosphate, orthophosphoric acid and triethylamine used for the preparation of mobile phase were of analytical grade (CDH, Chemicals Delhi, India). The membrane filters 0.22 µm and syringe filters 0.45 µm for the analysis was supplied by Millpores (Millipores Ltd. Banglore). Capsule formulations A (Milnace, Torrent Pharmaceuticals Ltd., India) and B (Milborn, Sun Pharmaceuticals Ltd., India) containing labeled amount of 50 mg of MIL were procured from local market.

Equipment

The Liquid Chromatographic procedures were carried out on Jasco make HPLC, (Jasco, Japan) equipped with Jasco PU 2080 binary gradient pumps, with variable wavelength programmable UV 2075 detector and Rheodyne injector with 20 µL fixed loop. Chromatographic integration and processing was carried out on Borwin software. A Puroshpere Star Hyber C18 column (5 µm, 4.6 x 250 mm id; Merck, Germany) was used as a stationary phase. The column eluents were monitored at 220 nm.

Preparation of mobile phase

The phosphate buffer was prepared by weighing 1.70 g of potassium dihydrogen phosphate and dissolving in 1000 mL of water (0.0125 M), to this 0.3 % of triethylamine was added. The pH of the solution was adjusted to 3.65 with the help of 0.1 M orthophosphoric acid. The mobile phase was prepared by mixing 720 mL of buffer and 280 mL of acetonitrile. Before use the mobile phase was filtered through 0.22 µm membrane filter.

Chromatographic conditions

The column was equilibrated with mobile phase and flow rate was maintained at 1.0 mL min^{-1} and eluents were monitored at 220 nm. The sample was injected using a 20 µL fixed loop. The column temperature was kept as ambient during the analysis.

Preparation of standard solutions

A stock solution of MIL was prepared by dissolving 100 mg of the drug in 100 mL volumetric flask with methanol. Aliquots of this solution were suitability diluted with mobile phase to get working standard solutions of MIL in the concentration range of 5-50 µg mL^{-1}.

Preparation of sample solution for assay

Twenty capsules of each brand were weighed and net content of each capsule was calculated. Capsule powder equivalent to 50 mg MIL was accurately weighed and transferred to a 100 mL volumetric flask with addition of about 80 mL of methanol. The mixture was sonicated for 30 min with shaking, and volume was made up to the mark with methanol. The above solution was centrifuged at 2500 RPM in the research centrifuge for 15 minutes and was filtered

through 0.45 µm syringe filter. The first 10 mL of the filtrate was rejected and subsequent filtrate was further diluted with mobile phase to obtain the solution of 20 µg mL^{-1}. The resulting solution was used as sample solution for assay and was analyzed as given under the described chromatographic conditions.

Method validation

The developed method was validated in terms of system suitability, specificity, linearity and range, precision, accuracy, limit of detection, limit of quantification, solution stability and robustness as per USP and ICH guidelines [10-11].

System suitability

The system suitability test was performed to ensure that the complete testing system was suitable for the intended application and it was performed by injecting the five replicate injections of standard preparation (20 µg mL^{-1}). The parameters measured were retention time, theoretical plates, asymmetry and peak area of MIL.

Specificity

Specificity is ability of an analytical method to measure the analyte free from interference due to placebo, blank and degradation products. It was estimated by spiking placebos of the capsule formulations into a pre weighed quantity of drug and were checked for the interference from the placebo and also from the blank i.e. diluent preparation which was injected separately. The specificity was also evaluated by forced degradation studies which ensure the selectivity of the proposed analytical method. The forced degradation studies were carried out as per ICH guidelines [12], by forcibly degrading MIL under different stress conditions such as hydrolytic, oxidative, thermal and photolytic degradation. Hydrolytic degradation was carried out by preparing MIL solutions (2 mg mL^{-1}) in 1 N HCL, 0.1 N NaOH and distilled water and refluxing at 100°C for 4 h. For oxidative studies MIL solution (2 mg mL^{-1}) was prepared in 3% hydrogen peroxide and was refluxed at 100°C for 4 h. Thermal degradation was carried out by exposing the MIL in petridish in hot air oven at 120°C for two days, similarly for photo degradation the aqueous solution MIL (2 mg mL^{-1}) was exposed to direct sunlight for 6 h. All the stressed samples were suitably diluted with mobile phase (acidic and basic hydrolytic stressed samples were appropriately neutralized) to get concentration 20 µg mL^{-1} and were injected on chromatographic system.

Linearity and Range

For evaluation of linearity the calibration curve was obtained at 10 concentration levels of MIL standard solutions 5-50 µg mL^{-1}. The solutions (20 µL) were injected in triplicate into liquid chromatographic system with chromatographic conditions previously given. For evaluation of linearity, peak area and concentrations were subjected to least square regression analysis to calculate calibration equation and correlation coefficient.

Precision and Intermediate Precision

Method precision studies were carried out by repeating the analysis of the standard solutions of 20 µg mL^{-1} six times on the same day under the same experimental conditions and reporting the %RSD values of the results obtained. The intra-day and inter-day precision study was carried out by estimating the corresponding responses of the analysis 3 times on the same day and on 3 different days (first, second and third day) and %RSD values were obtained. For precision by different analyst study, the same analysis procedure was performed by two different analysts and results were reported.

Accuracy

The accuracy was evaluated by the recovery of a known amount of MIL in synthetic mixture prepared by mixing MIL to placebo, to obtain concentrations of 80-120% of the labeled claim. The accuracy was calculated as the percentage of the drug recovered and also expressed as the percentage relative error between the measured mean concentrations and added concentration.

The Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD is the lowest concentration of an analyte that can reliably be differentiated from background levels. LOQ of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated from standard deviation of the response and the slope of the three linearity curves using the formula 3.3 α/S for LOD and 10 α/S for LOQ where α is standard deviation of response and S is mean of slope of three calibration curves. The LOQ was verified by injecting six replicates at its concentration at the LOQ level of MIL.

Robustness

The robustness of an analytical procedure refers to its ability to remain unaffected by small and deliberate variations in method parameter and provides an indication of its reliability for the routine analysis. The Robustness of the method was studied by making changes in the chromatographic conditions. In condition one the composition of the organic phase of mobile phase was varied by ± 5%. In the second condition the pH of the buffer preparation of the mobile phase was varied by ± 0.02 and finally in the third condition the flow rate was varied by ± 10% of the original flow rate.

Solution stability

The solution stability was also carried out to check the stability of the solution till 24 h and was performed by doing the analysis of solution at 0, 12 and at 24 h and comparing the results with the freshly prepared solution injected simultaneously.

RESULTS AND DISCUSSION

The present work describes development and validation of HPLC method for the determination of MIL in bulk drug and in the pharmaceutical capsule formulations.

Method development

The mobile phase for the assay of MIL was optimized and selected by taking different proportions of aqueous and organic phases which gave acceptable asymmetry and theoretical plates with appropriate run time. From the different mobile phases tried mobile phase consisting of phosphate buffer: acetonitrile (72: 28, v/v) was found to be satisfactory. However due to two amino groups present in the structure, the chromatogram displayed a tailing peak of MIL, which was avoided by adding 0.3 % v/v of triethyleamine. The pH of the mobile phase was also optimized since it is a basic drug, so ionization of the drug was found at the pH 3.65, where the drug gave symmetric and sharp peak for MIL at 1 mL min^{-1} flow rate with good theoretical plates and acceptable tailing factor. For quantitative analytical purpose wavelength was set at 220 nm, which provided better reproducibility with minimum interference. Under the chosen experimental conditions, the liquid chromatogram of MIL showed a single peak of the drug at retention time (Rt) 5.68 min with asymmetry of 1.29 (Figure 2).

System suitability

The system suitability was evaluated by calculating the %RSD values of peak area, retention time, asymmetry and theoretical plates of five standard replicates. The experimental results (Table 1) showed that the values were within the acceptable range indicating that the system was suitable for the intended analysis.

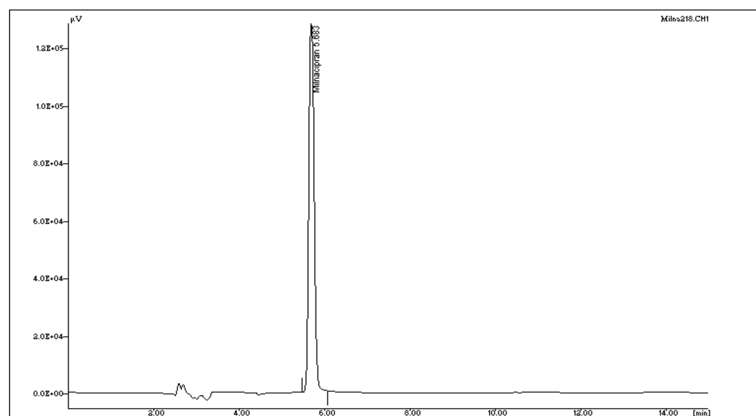

Fig. 2: HPLC chromatogram of MIL (20 µg mL-1) standard solution, Rt. 5.68 min.

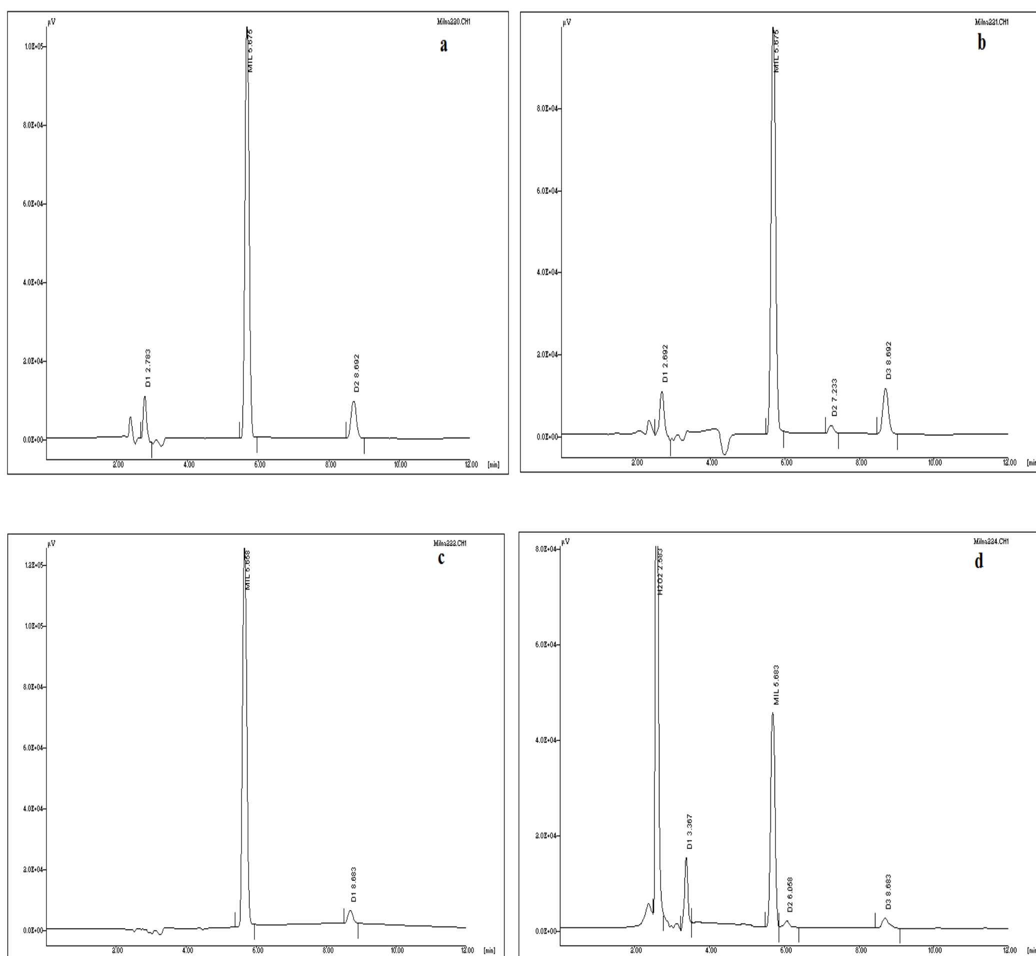

Fig. 3: HPLC Chromatogram of MIL (20 µg mL-1) a. Acid Degraded showing Rt of D1 at 2.78 min and D2 at 8.69 min. b. Base Degraded showing Rt of D1 at 2.69 min, D2 at 7.23 min and D3 at 8.69 min. c. Neutral degraded showing Rt of D1 at 8.68 min. d. Oxidative degraded showing Rt of D1 at 3.36 min, D2 at 6.05 min and D3 at 8.68 min. D1, D2, D3 are the degradation products of MIL.

Table 1: System suitability parameters of developed HPLC method for MIL.

Parameters	Observation*	%RSD
Peak Area	1003825	0.59
Retention Time (min)	5.68	0.21
Theoretical Plates	1413	0.78
Asymmetry	1.29	0.96

*Mean of five replicates

Specificity

In specificity study, standard solutions of MIL and the capsule placebo were injected and a single peak was obtained for MIL, which indicates that there was no interference from the excipients used and also from the mobile phase. The specificity study was also evaluated by examining the results of stress studies where the method is able to separate the main drug from the degradation products. Degradation studies were carried out using hydrolysis (acidic, basic, and neutral), chemical oxidation, thermolysis and photodegradtion. MIL was unstable in hydrolytic and oxidative studies and was stable in thermal and photolytic conditions of degradation. The result of forced degradation studies, with approximate % degradation and retention time of major degradation products is given in Table 2.

Figure 3. a, b, c, d. shows the HPLC chromatograms of hydrolytic and oxidative degradation of MIL which shows the MIL peak is well separated from all the degradation products formed during the different stress conditions. Thus specificity study ensures the selectivity of the developed analytical method which is able to separate and quantify MIL in presence of different degradation products.

Linearity and range

The linearity of the developed method was determined in triplicate at different concentrations ranging from 5-50 μg mL^{-1}. The regression analysis equation was $y = 49842x + 22115$ and correlation coefficient (r) was 0.9992, showing good linearity. The results confirmed the linearity of the standard curves over the range studied and the excellent reproducibility of the assay method.

Method precision and intermediate precision

Precision studies were carried out by repeating the analysis of the samples six times and the results shows that the mean assay value and %RSD are well within the acceptance criteria for the precision study. Similarly the results of intermediate precision study (intraday, interday and different analyst) were also determined for mean assay value and %RSD. The results of precision and intermediate precision are shown in Table 3.

Accuracy

Accuracy of the method was studied by applying the developed method to prepared synthetic mixtures of capsule excipients to which known amount of MIL corresponding to 80-120% of the label claim had been added. Mean recovery (Table 4) for MIL was between 99.21 and 100.12% indicating that the developed method was accurate for the determination of MIL in pharmaceutical formulation.

LOD and LOQ

LOD value was found to be 0.30 μg mL^{-1} and LOQ was 0.92 μg mL^{-1}. The LOQ was verified by performing six replicate analysis at its concentration, the %RSD of precision at LOQ was 1.93.

Robustness

The robustness of the method was evaluated by assaying the same sample under different analytical conditions deliberately changed from the original analytical condition. The results obtained were not affected by varying the conditions and were in accordance with the results for original conditions (Table 5). The %RSD value (1.32) of assay determined for the same sample under original conditions and with all the conditions of robustness indicates that the developed method was robust and not affected by deliberate changes in the method parameters.

Table 2: Results of stress degradation study of MIL

Stress Degradation condition	Approximate degradation observed (%)	Retention time of degradation products (min)
Acid Hydrolysis	8	2.78,8.69
Base Hydrolysis	11	2.69,7.23,8.69
Neutral Hydrolysis	4	8.68
Oxidative Degradation	30	3.36, 6.05, 8.68

Table 3: Results of method precision and intermediate precision study by the developed method for MIL

Precision Parameter		Mean Assay Value	% RSD
Method Precision [a]		99.56	0.96
Intraday Precision [b]	Initial After 3 hrs After 6 hrs	100.17	1.43
Interday Precision [b]	Day 1 Day 2 Day 3	99.30	1.18
Different Analyst Precision [b]	Analyst 1 Analyst 2	98.56	1.44

[a]n = 6, [b]n = 3

Table 4: Results from evaluation of the accuracy of the method

Level (%)	Amount of drug added (mg)	Amount of drug found (mg)	Mean recovery (%)*	% RSD
80	40.6 40.2 40.1	39.85 39.92 40.42	99.42	1.33
100	49.8 49.6 50.0	49.37 49.77 49.08	99.21	1.10
120	60.9 59.9 59.1	61.32 59.44 59.36	100.12	0.78

* Mean of three replicates

Table 5: Results of robustness studies for the determination of MIL by the developed method for MIL

Sr. No.	Parameter	Factor	Value	Retention time*	Asymmetric factor*
1	Change in organic phase	- 5 %	26.6	5.92	1.16
		0 %	28	5.68	1.12
		+ 5 %	29.4	5.37	1.13
			SD	**0.28**	**0.021**
2	Change in pH of buffer of mobile phase	- 0.02 %	3.63	5.51	1.15
		0.00 %	3.65	5.68	1.12
		+ 0.02 %	3.67	5.99	1.10
			SD	**0.24**	**0.025**
3	Change in flow rate of the method	-10.0 %	0.9	6.21	1.22
		0.0 %	1	5.68	1.12
		+10.0 %	1.1	5.15	1.16
			SD	**0.53**	**0.05**

*Mean of three replicates

Table 6: Assay results of MIL capsule dosage form using the proposed method

Formulation	Amount of drug taken (mg)	Amount of drug obtained (mg)*	% Recovery/Assay*
A	50.0	49.1 ± 0.29	99.3 ± 0.60
B	50.0	50.2 ± 0.37	101.0 ± 0.73

A Milnace (Torrent Pharmaceuticals Ltd. Ahmedabad, India), B Milborn (Sun Pharmaceutical Ltd. Baroda, India), *Mean value ± standard deviation of three determinations

Solution stability

The solution stability of the standard and the test sample solution was checked by analyzing both the solutions at interval of 12 h till 24 h at room temperature. The results showed that both the retention time and area of MIL was unchanged and no significant degradation was observed within the indicated period which was sufficient for performing analytical process.

Method application

The proposed validated liquid chromatographic method was successfully applied to the estimation of MIL in two different brands of pharmaceutical capsule formulations. The assay results obtained were satisfactory, accurate and precise as indicated by the good recovery and standard deviation (SD) values (Table 6). The developed method achieved rapid and accurate determination of MIL and can be used for the determination of MIL in drug substance and pharmaceutical preparation.

CONCLUSION

In proposed study, sensitive isocratic RP-HPLC method has been developed for determination of MIL. The developed method was validated and was found to be simple, sensitive, accurate and precise. The method was successfully used for determination of MIL in its pharmaceutical formulations. As the method separates the drug from its degradation products as well as all the degradation products from each other the method is stability indicating and can be conveniently used for routine quality control analysis of MIL in industries for batch release.

Future scope

The probable degradation pathways of the parent drug in different stressed conditions can be predicted by analyzing the stressed samples by LC-MS method, whereby molecular weights and the fragmentation results of the degradation products of MIL will confirm the most probable degradation pathways of the drug.

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