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Research Article

Optimized and Validated RP-HPLC Method for the Determination of Milnacipran Hydrochloride in Pharmaceutical Formulations

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ABSTRACT

A validated reverse phase high performance liquid chromatography (RP-HPLC) method has been developed and validated for the determination of Milnacipran hydrochloride in pharmaceutical preparations. Shimadzu HPLC containing SPD-20Av uv-visible detector, SIL 20 AC HT manual injector, LC-20 AT binary pump with software LC solution of version 1.2 was employed in the present study. Chromatography was carried out on a reverse phase C-18 column (250 x 4.6 mm x 5 µm length); optimum separation was achieved by using a mobile phase containing phosphate buffer (pH 3.6): acetonitrile at a ratio 70:30 with 1 mL/min flow rate and the detection was done at 220 nm. The retention time for Milnacipran hydrochloride was observed at 4.802 min. The method also produced linear responses in the concentration range from 25-75 µg/mL of Milnacipran hydrochloride with correlation coefficients of 0.999, accuracy of 101.3% and precision of 0.70%. As per ICH guideline, this developed method is fast, accurate, precise and sensitive hence it can be employed for routine analysis of Milnacipran hydrochloride in pharmaceutical formulations.

INTRODUCTION

Milnacipran (1-phenyl-1-diethylamino-carbonyl-2-amino-methylcyclopropane hydrochloride) hydrochloride (MCN) is an antidepressant drug characterized by serotonin and noradrenalin reuptake inhibitor (SNRI) and by virtual absence of postsynaptic effects [1]. Chemically, it is (1R, 2S)-rel-2-(aminomethyl)-N, N-diethyl-1-phenylcyclopropane carboxamide hydrochloride [2] (Fig. 1).

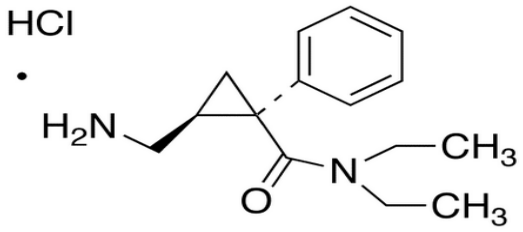

Figure 1: Structure of Milnacipran Hydrochloride

It has an empirical formula of $C_{15}H_{22}N_2O$-HCl and a molecular weight of 282.8 g/mol. MCN is synthesized, developed and marketed by Pierre Fabre Medicament.

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The indications for these medicines include treatment of depression, Generalized Anxiety Disorder (GAD), Social Anxiety Disorder (SAD), panic disorder and diabetic peripheral neuropathy (duloxetine only). The exact mechanism of the central pain inhibitory action of MC and its ability to improve the symptoms of fibromyalgia in humans are unknown [3]. The drug, which has no affinity for post-synaptic neurotransmitter receptors, was selected from a family of 1-aryl-2-aminomethylcyclopropane carboxylic acid derivatives for its potent inhibition of both noradrenaline and serotonin reuptakes [2]. In the treatment of major depression, MCN has achieved a similar efficacy to tricyclic antidepressants and a similar tolerability to selective serotonin re-uptake inhibitors [4].

From the literature review it reveals that very few UV spectrophotometric [4], LC-MS [5], U-HPLC [6], HPTLC [7] and HPLC methods [8-14] have been reported for the estimation of MCN in pharmaceutical dosage forms, laboratory prepared mixtures and biological matrices. Most of the reported chromatographic methods have tedious extraction procedures, time-consuming,

complex, multiple sample preparation steps, expensive and requires a skilled person to operate the instrument. Hence these methods are not suitable for routine analysis of MCN in quality control and quality assurance laboratories. Compared with the above mentioned chromatographic methods, spectrophotometry is considered as the most convenient analytical technique in many of the quality control and quality assurance laboratories because of its advantages, such as good selectivity, less expensive, simple instrumentation and less time consuming.

However, the purpose of the present study is to develop a simple, sensitive, accurate, precise, time and cost-effective and validated RP-HPLC method for the determination of MCN in pharmaceutical formulations. The developed method has been validated by evaluating system suitability, specificity, linearity, limits of detection and quantification, precision and accuracy according to ICH guidelines [15].

MATERIALS AND METHODS

Chemicals and Reagents

Pure MCN (Glenmark pharmaceuticals Pvt. Ltd.; India) used as working standard without further purification. A commercial MCN tablet was purchased from local market. HPLC grade acetonitrile (Merck, German) and potassium dihydrogen orthophosphate (Analytical reagent grade, Scharlau, Spain), orthophosphoric acid (Sigma-Aldrich, Germany), water for HPLC (PALL life sciences, India) were used for preparing the mobile phase. All other reagents used were of HPLC grade.

Chromatographic Conditions:

Instrumentation

A Shimadzu HPLC containing SPD-20Av uv-visible detector, a C18 reverse phase column (250 x 4.6 mm x 5 µm length), SIL 20 AC HT manual injector, LC-20 AT binary pump with software LC solution of version 1.2 was employed in the study.

Preparation of Mobile Phase

Phosphate buffer was prepared by dissolving 1.36 g of potassium dihydrogen orthophosphate in 1000 mL of distilled water and pH was adjusted to 3.6 with orthophosphoric acid. A freshly prepared 70:30 v/v mixture of buffer and acetonitrile solution was used as the mobile phase. The resulting solution was sonicated 5 min using ultrasonic bath and then filtered through 0.45 µm filter.

Preparation of Standard Stock Solution

About 25 mg of MCN working standard was dissolved with mobile phase and diluted up to 50 mL.

Preparation of Analytical Standard Solution

5 mL of this solution was diluted to 50 mL with mobile phase to make the concentration 50 µg/mL and filtered through a filter having a nominal pore size not greater than 0.45 µm.

Preparation of Assay Sample Solution

Twenty tablets were weighed accurately and grinded into fine powder. An amount of the powder equivalent to standard solution of MCN was weighed into a 50 mL volumetric flask and dissolved in about 25 mL of mobile phase and sonicated for 15 min in an ultrasonic bath. Cool the sample to room temperature. Finally diluted up to 50 mL. Then suitably diluted this solution with mobile phase to make the concentration 50 µg/mL. Filtered through a filter having a nominal pore size not greater than 0.45 µm. All solutions were stored at room temperature; these solutions were shown to be stable during the period of study.

Analytical Method Validation Parameters:

System Suitability Test

Before starting validation parameters, System Suitability must be established by injecting 20 µL each for six replicate injections of system suitability solution prepared as analytical standard solution. Using six peak areas, Relative Standard Deviation (RSD %), mean tailing factor were calculated.

Linearity and Range

The linearity was analyzed through the standard curves ranging from 25 to 75 $\mu g mL^{-1}$ by diluting appropriate amounts of MCN stock solution (500 $\mu g mL^{-1}$) with acetonitrile and prepared in triplicate. Three calibration curves were prepared in the same day. The linearity was evaluated by linear regression analysis, which was calculated by the least-square regression analysis.

Specificity

Specificity is determined by injecting separately blank, placebo, standard and sample solution of MCN in duplicate.

Precision

Precision was done by repeatability or intra-assay precision and intermediate precision

(inter-day precision) of both standard and sample solutions. Precision was determined in six replicates of both MCN standard solution (200 μgmL^{-1}) and sample solution (200 μgmL^{-1}) on the same day (intra-day precision) and daily for 6 times over a period of one week (inter-day precision). The results were expressed as %RSD of the measurements.

Accuracy

To check the accuracy of developed method and to study the interference of formulation additives analytical recovery experiments were carried out by standard addition 80%, 100% and 120% of the label claim. Accuracy was conducted by adding known amounts of MCN to the sample matrix and three different concentrations of test sample were prepared. Duplicate injections were made for each concentration level.

Robustness

The robustness of this validation was conducted by changing two different parameters (Temperature: 25°C and 30°C and flow rate: 1 mL/min and 0.8 mL/min) of the method by using the same concentration of test sample of repeatability sample.

RESULTS AND DISCUSSIONS

Analytical Method Validation:

System Suitability

Chromatograms were automatically integrated and visually inspected for an acceptable integration. The relative standard deviation of the peak areas (%RSD 0.074). All other parameters are shown in Table 1. The system suitability parameters were within the limits.

Linearity and Range

A good linear relationship (r^2=0.999) was observed between the concentration of MCN and the respective ratio of peak areas (Table 2). The regression curve was constructed by linear regression fitting and its mathematical expression was y=44800x+2475 (where y is the ratio of peak areas of the drug to that of reference standard and x is the concentration of MCN). The lower limit of quantitation (LLOQ) was defined as the lowest concentration within the linear range (25.0 μg/mL). The upper limit of quantitation (ULOQ) was defined as the highest concentration within the linear range (75.0 μg/mL).

Table 1: System Suitability Test

Parameter	Value (mean±%RSD)
Peak area	2248001±0.074
Tailing factor	1.522±0.002
Theoretical plate	19536±8.183
Retention time	4.802±0.051

Specificity

A typical HPLC chromatogram of blank mobile phase (a), placebo (b), MCN standard preparation (c) and MCN test sample (d) are shown in Fig. 2. The HPLC chromatograms recorded for blank and the mixture of the inactive ingredients revealed no peaks within retention time around 4.802 min, and the peak purity was 99.99%.

Precision

The values of %RSD for intraday and inter-day variation are given in Table 4. The repeatability (data presents in Table 3) and intermediate precision study of the developed method demonstrate %RSD 0.895 for analyst-1 and %RSD 0.463 for analyst-2 where %RSD value for 12 samples was 0.679 which were not more than 2.0 % indicating the developed method has excellent repeatability and intermediate precision.

Table 2: Linearity and range

% of Nominal value	Conc. Of Standard (μg/mL)	Peak area	Statistical Analyses
50%	25.00	1123592	Regression correlation coefficient (R^2)= 0.999 y-intercept = 2475 Slope of regression line = 44800
60%	30.50	1359850	
80%	40.60	1796444	
100%	50.80	2241103	
120%	61.00	2712881	
140%	71.10	3166458	
150%	75.00	3359908	
Lower limit of quantitation (LLOQ)			25.00 μg/mL
Upper limit of quantitation (ULOQ)			75.00 μg/mL

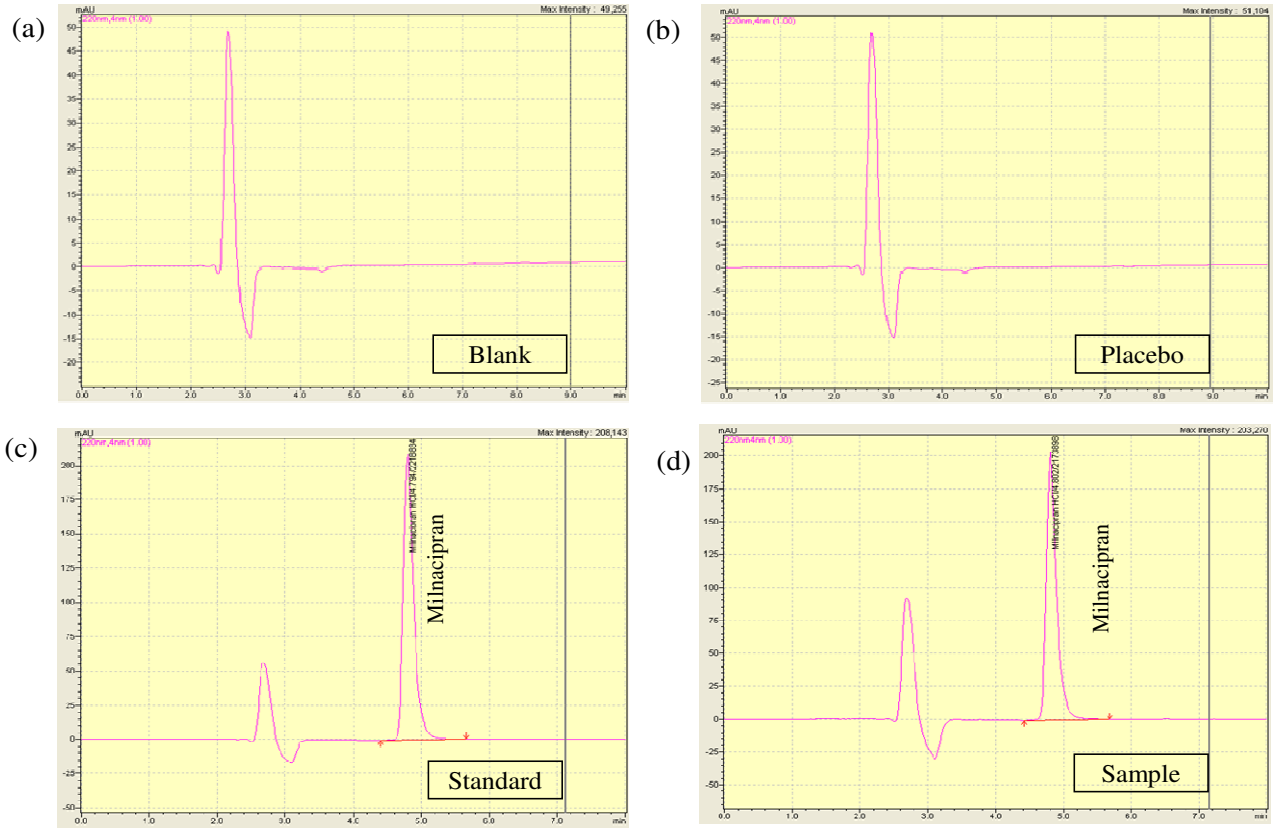

Figure 2: Typical Chromatogram of (a) Blank (b) Placebo (c) Milnacipran Hydrochloride Standard Preparation and (d) Milnacipran Hydrochloride Test Sample.

Table 3: Repeatability

Sample	Peak area of Sample	Average peak area of Sample	Assay (%)	%RSD	
				Result	Limit
1	2109268	2106108	98.48	0.895	NMT 2.0%
	2102948				
2	2168976	2168298	98.16		
	2167620				
3	2211326	2212503	98.04		
	2213679				
4	2241041	2242036	98.92		
	2243030				
5	2215611	2216092	99.91		
	2216573				
6	2271588	2271389	100.1		
	2271191				
Average of Assay (%)			98.94 ± 0.87		

Accuracy

The overall results of percent recoveries (mean±%RSD) of MCN in pure and drug-matrix solutions are demonstrated in Table 5. The validity and reliability of proposed method was assessed by recovery studies by standard addition method. The mean of % recovery 101.3%; and % RSD 0.10% were found to be within limit (NMT 2%) for the developed method [14]. This result revealed that any small change in the drug concentration in the solution could be accurately determined by the developed analytical method.

Table 4: Intermediated Precision

Analyst-1				Analyst-2		
Sample	**Peak area of Sample**	**Average peak area of Sample**	**Assay (%/Tablet)**	**Peak area of Sample**	**Average peak area of Sample**	**Assay (%/Tablet)**
1	2109268	2106108	98.48	2154806	2155132	99.00
	2102948			2155457		
2	2168976	2168298	98.16	2157335	2149927	98.08
	2167620			2142518		
3	2211326	2212503	98.04	2169168	2165138	98.76
	2213679			2161108		
4	2241041	2242036	98.92	2173784	2174345	99.24
	2243030			2174906		
5	2215611	2216092	99.91	2148166	2147387	99.21
	2216573			2146608		
6	2271588	2271389	100.10	2234649	2233374	98.44
	2271191			2232099		
%RSD for analyst-1			0.895	**%RSD for analyst-2**		0.463
Mean %RSD			0.679%			

Table 5: Accuracy Studies

% of Nominal Value	**Peak area of Sample**	**Average peak area of sample**	**%Recovery**
80%	1842965	1843074	101.2
	1845449		
100%	1840808	2352614	101.2
	2352378		
120%	2352124	2745664	101.4
	2353339		
Mean±%RSD			101.25±0.10

Table 6: Robustness of the Method

Temperature (C)	**Flow rate (mL/min)**	**Assay, (%/Tablet)**	**% RSD**	**Tailing Factor**	**Theoretical plate**
25	1	98.52	0.138	1.518	20423
25	0.8	98.04	0.148	1.484	17559
30	1	98.20	0.074	1.545	21172
30	0.8	98.95	0.152	1.002	18993

Robustness

Robustness of the method was found out by testing the effect of deliberate changes in the chromatographic conditions and the corresponding peak areas. The factors selected for this purpose were temperature and flow rate. The %RSD of robustness testing under different altered conditions is given in Table 6. The method was found to be robust enough that the RSD of peak area, tailing factor were not apparently affected by variation in the chromatographic conditions.

CONCLUSION

The developed RP-HPLC method for quantitative determination of MCN is simple, precise, accurate, reproducible and highly sensitive. The developed method was completely validated and satisfactory results were obtained as per ICH guidelines. Therefore, the proposed method can be used for routine analysis of MCN in pure and pharmaceutical dosage forms.

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CONFLICT OF INTERESTS
The authors declare no conflict of interest.

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