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A Validated Rp-Hplc Method for the estimation of Milnacipran in Tablet dosage forms

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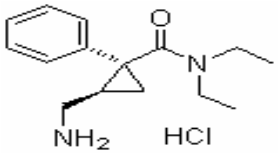
Abstract : A simple, selective, rapid, precise and economical reverse phase HPLC method has been developed for the determination of Milnacipran in tablet. The analyte was resolved by using a disodium hydrogen phosphate buffer 40mM containing 0.1%triethylamine (adjusted to pH 4.8 by orthophosphoric acid) and Methanol in the ratio of 75:25, v/v). On an isocratic HPLC system (PEAK) consisting UV lamp visible detector, ODS C-18, RP column (150 mm x 4.6 mm i.d., 5µm) at a wavelength of 210 nm. The linear dynamic range for Milnacipran was 2.0 µg/mL – 12.0µg/mL. The limit of detection [LOD] and Limit of quantification [LOQ] for Milnacipran was 0.5µg/mL and 1.5µg/mL respectively.
Key words: Milnacipran, HPLC, linearity, validation.

INTRODUCTION:

Milnacipran is chemically designated as (1R*, 2S*)-2-(aminomethyl)-N, N-diethyl-1-phenylcyclopropane carboxamide. Milnacipran is the first in a new class of serotonin-norepinephrine reuptake inhibitor (SNRI)[1,2] a new medication Milnacipran hasben shown to be very effective at treating chromic paid conditions, and currently being evaluated for treatment of the fibromyalgia syndrome was shown to prove. Literature survey reveals that a very few HPLC methods [3-8] has been reported for the estimation of Milnacipran. In the present investigation a new RP HPLC method has been reported for the estimation of Milnacipran form tablet dosage form. The present work describes 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 [9]. The chemical structure of the Milnacipran is as follows:

Fig.1, Chemical structure of Milnacipran



EXPERIMENTAL

1. Chemicals and materials:

The pharmaceutical grade pure sample of Milnacipran (99.28%) was procured from CELON Laboratories limited, Andrapradesh. Acetonitrile solvent of

analytical grade was obtained from E Merck Ltd, Mumbai, India. Orthophosphoric acid AR grade were procured from Qualigens Fine Chemicals, Mumbai, India. The HPLC grade water was obtained from a Milli-QRO water purification system, sonicated and used.

2. Instrumentation:

The development and validation of the method was performed on a isocratic HPLC system (PEAK) consisting of Isocratic liquid pump, LC 8200 variable wavelength UV detector with Millennium® version 32 software on a Dell computer. The analytical column used to achieve chromatographic separation was a stainless steel ODS- C- 18 RP column (150 mm x 4.6 mm i.d.,5µm) purchased from Waters Corporation (Bedford, MA, USA) protected by a guard column of the same material.

3. Standard stock solution:

An accurately weighted sample of 10 mg of Milnacipran was dissolved in methanol to give standard stock solution of 100µg/ml. A series of working standard solutions (2.0µg/mL - 12µg/mL were obtained by diluting the stock solutions with mobile phase consisted of disodium hydrogen phosphate buffer 40mM containing 0.1%triethylamine (adjusted to pH 4.8 by orthophosphoric acid) and Methanol in the ratio of 75:25, v/v). All the volumetric flasks containing Milnacipran were wrapped with aluminium foil and stored in the dark.

4. Preparation of tablets containing the drug:

An average of ten tablets of Milnacipran(Dalcipran) were weighed and ground to fine powder. Accurately weighed powder sample equivalent to 250 mg of Milnacipran was dissolved in methanol in a 100mL volumetric flask. The flask was placed in an ultrasonic bath at room temperature for 10min. After sonication, the solution was allowed to stand for 5.0 min. 1.0mL was transferred into a 100 ml volumetric flask and diluted to the mark with mobile phase. A sample of 0.5µLof this solution was directly injected. The average content of the tablets was determined either from the calibration graph or using the corresponding regression equation.

5. Chromatographic conditions:

The mobile phase was filtered by passing through a 0.45µm membrane filter (Millipore, Bedford, MA, USA).Chromatographic analysis was carried out at ambient temperature. The mobile phase consisted of disodium hydrogen phosphate buffer 40mM containing 0.1%triethylamine (adjusted to pH 4.8 by orthophosphoric acid) and Methanol in the ratio of 75:25, v/v). The flow rate was 1.0mL/min. The effluent was monitored spectrophotometrically at a wavelength of 210nm. The optimized chromatographic conditions for the determination of Milnacipran are represented in Table.1.

Table.1 Optimized chromatographic conditions

Chromatographic Parameters	Peak Conditions
Mobile phase	Disodium hydrogen phosphate buffer 40mM containing 0.1%triethylamine (adjusted to pH 4.8 by orthophosphoric acid) and Methanol in the ratio of 75:25, v/v.
Column	Inertsil ODS C_{18} column (150mm x 4.6mm i.d., 5µm)
Flow rate	1.0mL.min^{-1}
Detection	PDA : 210nm
Injection volume	10 µL
Temperature	ambient temperature 25±2 °C
Retention time	15.511 minutes
Run time	40 minutes
Area	15620582 mAU
pH	4.8
Pressure	17-20 Mpa

Table .2 Calibration of the RP HP LC for the estimation of Milnacipran

Concentration (µg)	**Area (mAU)**
2.0	15620582
4.0	31241164
6.0	46500000
8.0	62482328
10.0	80500000
12.0	93623492
Regression equation :	Y = a X + b
Slope (a) :	8E+06
Intercept(b) :	309217
Correlation coefficient :	0.9994

Fig.2. Typical HPLC chromatogram of standard solution of milnacipran

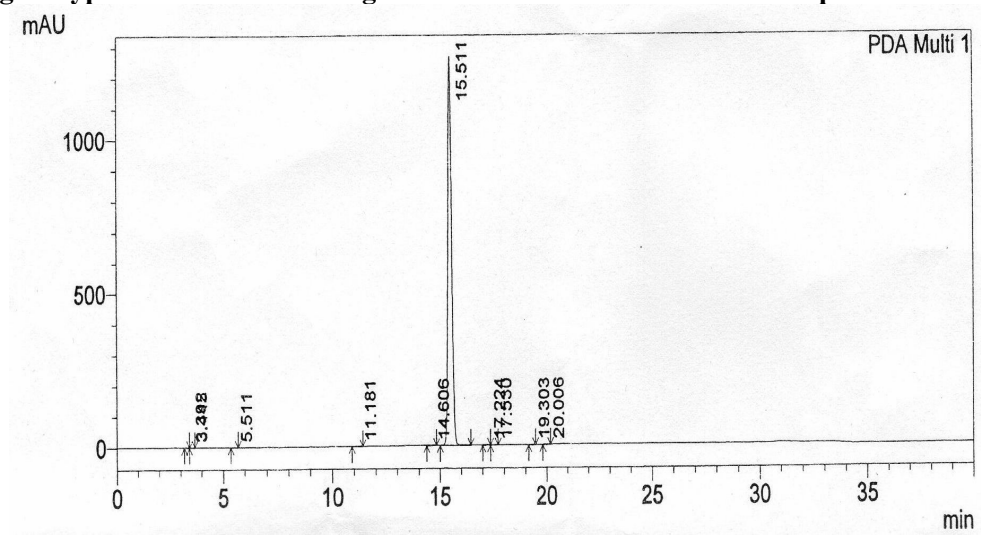
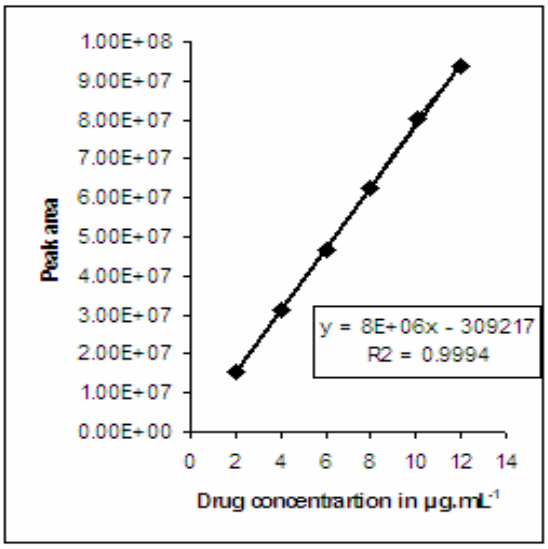


Fig.3. Calibartion Curve of Milnacipran



RESULTS AND DISCUSSION

A) METHOD DEVELOPMENT:

Several tests were performed in order to get satisfactory separation-resolution of Milnacipran in different mobile phases with various ratios by using C18 column. The mobile phase consisted of disodium hydrogen phosphate buffer 40mM containing 0.1%triethylamine (adjusted to pH 4.8 by orthophosphoric acid) and Methanol in the ratio of 75:25, v/v). The mobile phase was freshly prepared and filtered through a 0.45μm membrane filter (Millipore, Bedford, MA, US) and degassed by an ultrasonic bath. The injections were carried out through a 10.0μL loop. The analytes were detected and quantified by UV detection at a wavelength of 210nm.

B) METHOD VALIDATION:

I) Linearity:

The linearity for HPLC method was determined at eight concentration levels ranging from 2.0 - 12.0µg.mL^{-1} for Milnacipran**.** The calibration curve was constructed by plotting response factor against concentration of Milnacipran (Fig.3). The slope and intercept value for calibration curve were y = 8E+06X-309217 (R2= 0.9994) for Milnacipran, where **Y** represents the ratio of peak area ratio of analyte and **X** represents analyte concentration. The results were satisfactory shown that significant correlation exists between response factor and concentration of drug within the concentration range indicated on Y-axis (**Table.2)**.

ii)Sensitivity:

The Limit of Detection (LOD) was determined as lowest concentration giving response and Limit of Quantification (LOQ) was determined as the lowest concentration analyzed with accuracy method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The Limit of Detection (LOD) and the Limit of Quantification (LOQ) for Milnacipran was found to be 0.5µg.mL^{-1} and 1.5µg.mL^{-1} respectively.

iii)Precision:

The precision of the method was demonstrated by interday and intraday variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the interday variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug peaks and percentage RSD were calculated and presented in Table.3. From the data obtained, the developed RP-HPLC method was found to be precise.

Table 3.Results from intra-day and inter-day precision experiments

Drug	Actual concentration (µg.mL^{-1})	Intra-Day			Inter-Day		
		Found*,µg.mL^{-1}±SE	%RSD	%Bias	Found*,µg.mL^{-1}±SE	%RSD	%Bias
Milnacipran	**4**	4.01 ± 20.26	1.99	-0.25	4.03 ± 20.56	2.03	-0.75
	8	8.03 ± 4.42	0.87	-0.37	8.06 ± 5.01	0.99	-0.75
	12	12.01 ± 3.46	1.02	-0.08	12.07 ± 5.58	1.65	-0.58

Table.4 Recovery studies of the proposed HPLC method

Drug studied	HDH in tablet (µg.mL^{-1})	Pure HDH added (µg.mL^{-1})	Total HDH found ± SD*, µg.mL^{-1}	%HDH recovered
Milancipran	**6.0**	**3.0**	8.95 ± 0.22	98.33
	6.0	**6.0**	11.99 ± 0.17	99.83
	6.0	**9.0**	15.15 ± 0.24	101.7

All the values are the averages of three determinations

Table.5 Results of analysis of tablet containing Milnacipran & recovery studies

Pharmaceutical formulation	Amount of Milnacipran		% of recovery
	labelled	found	
Dalcipran	250mg	248.9mg	99.56%

All the values are the averages of three determinations

IV) Accuracy [Recovery studies]:

To further assess the accuracy of the method, recovery experiments were performed by applying the standard-addition technique. The recovery was assessed by determining the agreement between the measured standard concentration and added known concentration to the sample. The test was done by spiking the pre-analyzed tablet powder with pure milnacipran at three different levels [50, 100 and 150 % of the content present in the tablet powder (taken)] and the total was found by the proposed method. Each test was repeated three times. In all the cases, the recovery percentage values ranged between 98.33 and 101.7. Closeness of the results to 100 % showed the fairly good accuracy of the method. The results are shown in Table 4.

v) Ruggedness and Robustness

Ruggedness test was determined between two analysts, instruments and columns. Robustness of the method was determined by small deliberate changes in flow rate, mobile phase pH and mobile phase ratio. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was rugged and robust.

CONCLUSION

The HPLC method developed in this study has the sensitivity, selectivity, reproducibility, and stability which make it versatile and valuable in many applications, specifically in pharmacokinetic studies, kinetics study of Milnacipran in pharmaceutical dosage form and for drug level monitoring. The method can also be readily adapted to routine quality control analysis.

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