Asian Journal of Pharmaceutical Technology & Innovation
ISSN: 2347-8810

Research Article

Received on: 12-07-2015
Accepted on: 17-11-2015
Published on: 15-12-2015

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Method Development and Validation of Milnacipran by using RP-HPLC Method

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ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Milnacipran, in its pure form as well as in capsule dosage form. Chromatography was carried out on a phenomanix C_{18} (4.6 x 250mm, 5µm) column using a mixture of Acetonitrile: Triethylamine buffer pH 4.5(65:35% v/v)as the mobile phase at a flow rate of 0.8ml/min, the detection was carried out at 215 nm. The retention time of the Milnacipran was 2.16 min respectively. The method produce linear responses in the concentration range 5-25 mg/ml of Milnacipran. The method precision for the determination of assay was below 2.0% RSD. The method is useful in the analysis of bulk and pharmaceutical formulations of Millancipran.

Key-words: Milnacipran, RP-HPLC, validation.

Cite this article as:
Palleppagari Sridhar, K. Venkateswar Rao, C. Radhika, A. Sujitha, V. Uma Maheswara Rao, Method Development and Validation of Milnacipran by RP-HPLC Method, Asian Journal of Pharmaceutical Technology & Innovation, 03 (15); 2015.*
www.asianpharmtech.com

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 C15H23ClN2O and molecular weight 282.81.Thereare 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.

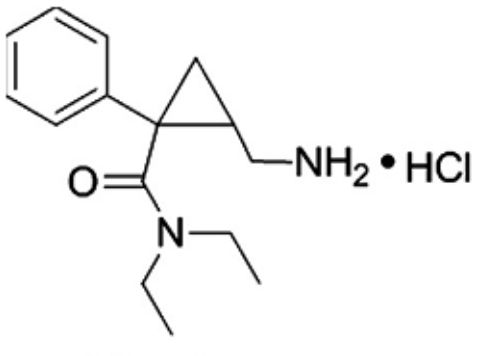


Fig 1 : Chemical structure of Milnacipran

MATERIALS AND METHODS

Chemicals and instrumentation:

Milnacipran hydrochloride (purity 99.80 %) was obtained as a gift sample from Sura labs Hydarabad, 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)

Table1:Chemicals and instrumentation

	Chemical	Brand names
1	Milnacipran	Sura labs
2	Water and Methanol for HPLC	LICHROSOLV (MERCK)
3	Acetonitrile for HPLC	Merck
4	Triethylamine	Merck
5	Ortho Phosphoric acid	Merck

Analytical Method Validation [15]:

VALIDATION PARAMETERS

- **Accuracy**

The accuracy of measurement is defined as the closeness of the measured value to the true value. In a method with high accuracy, a sample (whose "true value" is known) is analyzed and the measured value should ideally be identical to the true value. Typically, accuracy is represented and determined by recovery studies but there are three ways to determine accuracy:

1. Comparison to a reference standard
2. Recovery of the analyte spiked into blank matrix or
3. Standard addition of the analyte.

The ICH documents recommended that accuracy should be assessed using a minimum of nine determinations over a minimum of three concentrations levels the specified range (i.e., three concentrations and three replicates of each concentration). Accuracy was tested (%Recovery and %RSD of individual measurements) by analyzing samples at least in triplicate, at each level (80%, 100% and 120% of label claim) is recommended. For each determination fresh samples were prepared and assay value is calculated.

Acceptance criteria: The accuracy should be within 98-102%.

Precision

Precision can be defined as "the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogeneous sample". A more comprehensive definition proposed by the International Conference on Harmonization (ICH) divides precision into three types:

- Repeatability
- Intermediate precision
- Reproducibility

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision and involves multiple measurements of the same sample (different preparations) by the same analyst under the same conditions. At least

1. 5 or 6 determinations
2. At two or three different concentrations

Should be done and the relative standard deviations were calculated. The % RSD can be calculated by,

$$\% \textbf{ RSD} = \frac{\textbf{SD}}{\textbf{MEAN}} \textbf{X100}$$

Where, RSD = relative standard deviation

SD = standard deviation

The standard deviation SD is given by,

$$\sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(x_i - x)^2}$$

Intermediate precision: It is the agreement of complete measurements (including standards) when the same method is applied many times within the same laboratory. Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment etc.

Reproducibility: Reproducibility expresses the precision between laboratories and is often determined in collaborative studies or method transfer experiments.

The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

Acceptance criteria: The % RSD should be less than 2.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample.

It is a measure of how well a calibration plot of response vs. concentration approximates a straight line. Linearity can be assessed by performing single measurements at several analyte concentrations. The data are then processed using a linear least squares regression. The Resulting plot slope, intercept and correlation coefficient provide the desired information on linearity.

Acceptance criteria: Correlation coefficient (R^2)=1 (or) ≈0.999.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present; these include impurities, degradants and matrix etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedures. This definition has the following implications:

Identification: To ensure the identity of an analyte.

Purity Tests: To ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content etc.

Assay (content or potency): To provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample .

Acceptance criteria: No interference should be present.

Limit of Detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. LOD can be defined as the smallest level of analyte that gives a measurable response. The detection limit is usually expressed as the concentration of the analyte (percentage parts per million) in the sample. It is usually determined by 3 ways:

1. Based on Visual Evaluation
2. Based on Signal-to-Noise
3. Based on the Standard Deviation of the Response and the Slope

The limit of detection may be expressed as

$$\mathbf{LOD} = \frac{\mathbf{3.3\sigma}}{\mathbf{S}}$$

Where, σ = the standard deviation of the response

S = the slope of the calibration curve.

Acceptance criteria:S/N should be 3:1

Limit of Quantitation

The quantitation limit of an analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. LOQ is usually expressed as the concentration of the analyte (percentage parts per million) in the sample. . It is usually determined by 3 ways:

1. Based on Visual Evaluation
2. Based on Signal-to-Noise
3. Based on the Standard Deviation of the Response and the Slope

The limit of Quantitationmaybe expressed as:

$$\mathbf{LOQ} = \frac{\mathbf{10\sigma}}{\mathbf{S}}$$

Where, σ = the standard deviation of the response

S = the slope of the calibration curve.

Acceptance criteria: S/N should be 10:1

Robustness

It is defined as a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It involves a number of method parameters that are varied within a realistic range and the quantitative influence of the variables is determined. The typical variations are:

- Influence of variations of pH in a mobile phase.
- Influence of variations in mobile phase composition.
- Different columns (different lots and/or suppliers).
- Temperature.
- Flow rate.

System Suitability Testing

Prior to the analysis of samples each day, the operator must establish that the HPLC system and procedure are capable of providing data of acceptable quality. This is accomplished with system suitability experiments, which can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision.

The parameters that can be used to determine system suitability prior to analysis, includes Plate number (N), Tailing factor, k and/or α, Resolution (Rs) and Relative standard deviation (RSD) of peak height or peak area for repetitive injections. Typically at least two of these criteria are required to demonstrate system suitability for any method Robustness.

RESULTS AND DISCUSSION

HPLC METHOD DEVELOPMENT: Selection of Initial Conditions for Method Development:

A. Determination of Solubility of Drug:

Solubility of Milnacipran[20,22,23]

Taken small amount of sample and dissolved it in various solvents and the solubility of drugs. Was checked which is shown in below table

Solvents	Milnacipran
Methanol	Soluble
Ethanol	Freely soluble
Water	Sparingly soluble
Acetonitrile	Freely soluble

B. Determination of Detection Wavelength by UV Spectroscopy:

Accurately weighed and transferred about 100mg of Milnacipran working standard into a 100mL volumetric flask separately, then added to it 100mL of methanol and sonicated for 10 minutes to dissolve. From this, standard stock solution (1mg/mL) 1ml was transferred into 10mL volumetric flask and volume was made up to 10ml with methanol to get concentration of 100µg/mL. Finally diluted 1mL of above solution to 10mL using methanol and mixed well. The concentration of the working solution thus produced was 10µg/mL. The working standard solutions of Milnacipran (10µg/mL) were scanned over the range of 200-400nm using blank. By observing the overlain spectra, λ 215 nm was taken as absorption maximum and at this wavelength trials were done.

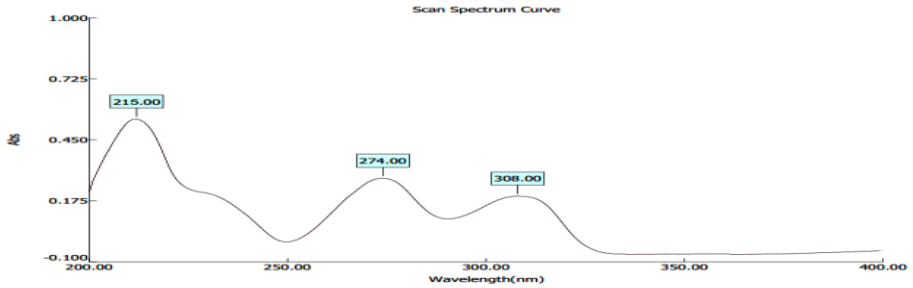


Fig 2 : UV Spectrum

C. Selection of Chromatographic Methods:

The proper selection of methods depends upon the nature of the sample (ionic or ionisable or neutral molecule) its molecular weight and stability. The drugs selected are polar, ionic and hence Reversed phase chromatography was carried out on a phenomanixC_{18} (4.6 x 250mm, 5µm) column using a mixture of Acetonitrile: Triethylamine buffer pH 4.5(65:35% v/v)as the mobile phase at a flow rate of 0.8ml/min, the detection was carried out at 215nm. The retention time of the milnacipranwas 2.16 min respectively.

Table 3: Peak results for assay standard

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Milnacipran	2.168	515979	479316	5814	1.42
2	Milnacipran	2.165	515894	454466	5810	1.49
3	Milnacipran	2.173	515628	477915	5817	1.49
4	Milnacipran	2.176	516479	481267	5849	1.50
5	Milnacipran	2.180	517729	482601	5857	1.44
Mean			516341.8			
Std.Dev.			834.387			
%RSD			0.161596			

Assay (Sample):

Table:4 Peak results for assay sample

S.No	Name	RT	Area	Height	USPTailing	USPPlateCount	Injection
1	Milnacipran	2.177	526961	463878	1.49	5820	1
2	Milnacipran	2.184	535275	469090	1.50	5810	2
3	Milnacipran	2.186	520057	463262	1.52	5841	3

Table 5 :Results of repeatability for Milnacipran

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Milnacipran	2.198	517041	449653	5806	1.55
2	Milnacipran	2.194	514537	444489	5803	1.60
3	Milnacipran	2.190	512545	440821	5785	1.60
4	Milnacipran	2.197	515013	447677	5778	1.56
5	Milnacipran	2.192	516855	433328	5777	1.60
Mean			515198.2			
Std.dev			1847.421			
%RSD			0.358584			

Table 6: Results of Intermediate precisionfor Milnacipran

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count Count	USP Tailing
1	Milnacipran	2.147	517716	513471	5737	1.53

2	Milnacipran	2.128	519199	442971	5783	1.54
3	Milnacipran	2.131	511959	444851	5770	1.54
4	Milnacipran	2.138	518569	434642	5797	1.58
5	Milnacipran	2.130	513071	431357	5753	1.57
6	Milnacipran	2.138	512531	423554	5749	1.57
Mean			515507.5			
Std.Dev.			3324.616			
%RSD			0.358584			

LINEARITY

Table 7: concentration V/s peak area

Concentration µg/ml	Average Peak Area
0	0
5	168581
10	354709
15	522589
20	696532
Correlation Coefficient (r)	0.999

LINEARITY PLOT:

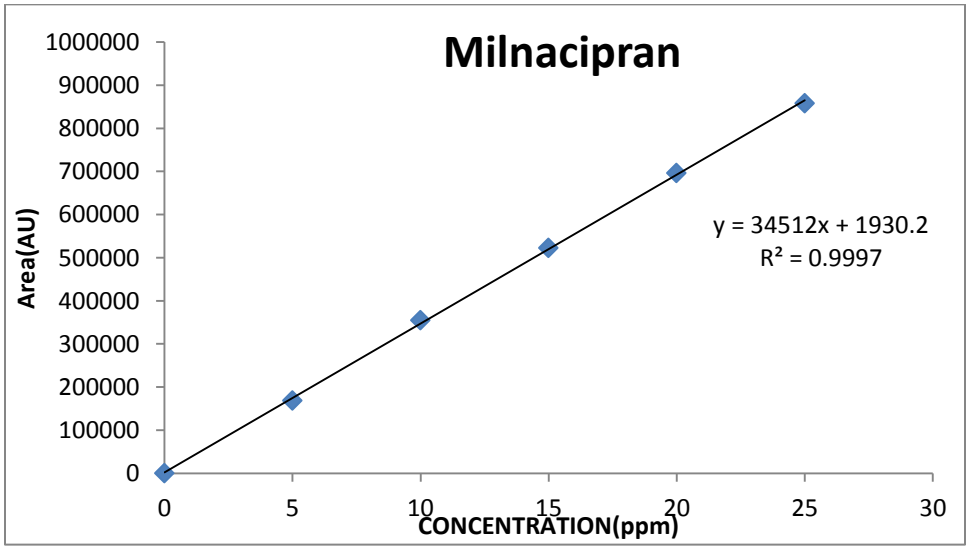


Fig 3: Linearity Graph

LIMIT OF DETECTION FOR MILNACIPRAN

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Quantitation limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

where

σ = Standard deviation of the response

S = Slope of the calibration curve

Robustness

The robustness was performed for the flow rate variations from 0.7 ml/min to 0.9ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for milnacipran. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±10%. The standard and samples of milnacipran were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table 8: Robustness results

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Flow rate of 0.8 ml/min	483391	2.163	5839	1.43
Flow rate of 0.7 ml/min	548389	2.897	5626	1.28
Flow rate of 0.9 ml/min	485995	1.929	5732	1.33
More aqueous phase (about 5 % Increase in Buffer)	624528	2.444	5531	1.50
More aqueous phase (about 5 % Increase in Acetonitrile)	599681	2.275	5740	1.50

Fig 4 :Optimized Chromatogram (Standard)

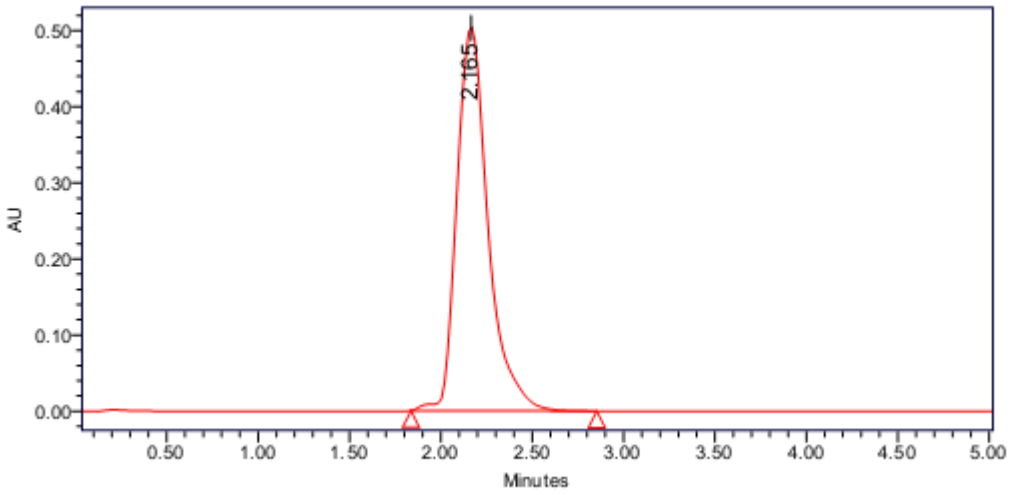
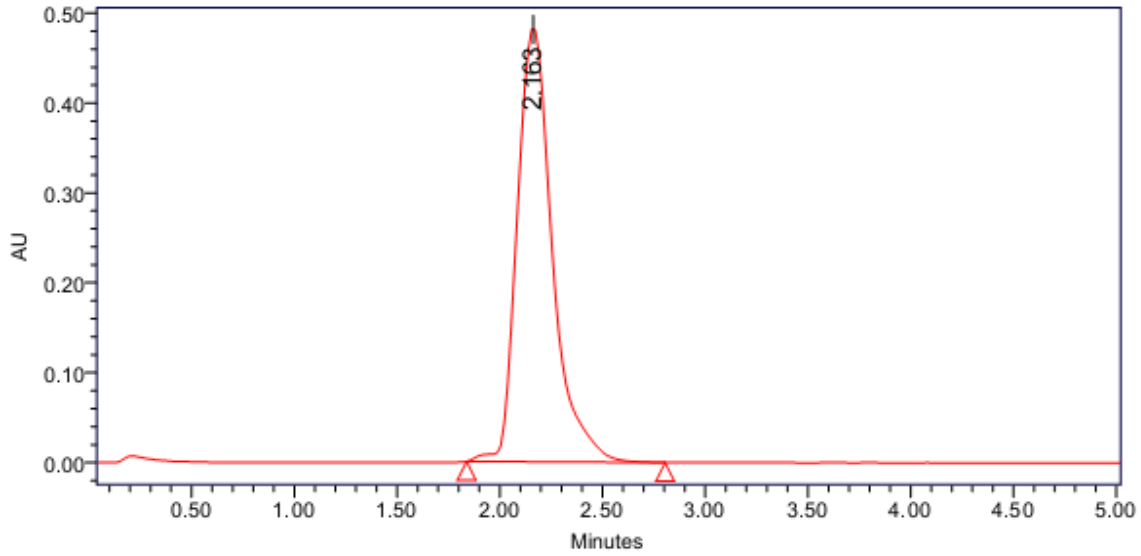


Fig5: Optimized Chromatogram (Sample)



SUMMARY AND CONCLUSION

RP-HPLC method was developed for estimation of Milnacipranr in bulk and Pharmaceutical dosage form. Chromatographic separation was, carried out on a Phenomenex C18 (4.6×250mm) 5µ column with mobile phase comprising mixture of Acetonitrile: Triethylamine buffer pH4.65 Methanol (65:35) at a flow rate of 0.8ml/min, the detection was carried out at 215nm

Table9 :Summary for RP-HPLC Method

S.NO	PARAMETER	ACCEPTANCECRITERIA	RESULTS OBTAINED FOR Milnacipran
1	System suitability	Theoretical Plates-NLT 2000	5827
		Tailing factor-NMT 2	1.5
		Retention time	2.1
2	Precision	%RSD- NLT 2	0.35
3	Linearity	Correlation Coefficient NLT 0.999	0.999
4	Accuracy	Percentage Recovery 98-102%	99.68
5	Limit of detection		0.71 µg/ml
6	Limit of quantitation		2.17 µg/ml

9. CONCLUSION

The proposed RP-HPLC method was found to be precise, specific, accurate, rapid and economical for estimation of Milnacipranin bulk and Pharmaceutical dosage form. The sample recoveries in all formulations were in good agreement with their respective Label Claims and the % RSD values were with in 2 and the method was found to be precise. This method can be used for routine determination of Milnacipran in bulk and in Pharmaceutical dosage forms.

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