



Spectrophotometric Determination of Nor-Epinephrine Serotonin Reuptake Inhibitor (SNRI) Drug Milnacipran in Pure and in Dosage Forms

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Milnacipran hydrochloride is an antidepressant drug belonging to the class of serotonin and nor-adrenaline reuptake inhibitors. Rapid and cost effective UV-spectrophotometric methods were developed for the estimation of milnacipran hydrochloride in bulk, pharmaceutical formulations and *in vitro* release samples. Milnacipran hydrochloride was estimated at 220 nm wavelength in the phosphate buffer (pH = 6.8 buffer) and hydrochloric acid buffer (pH = 1.2 buffer). Linearity range was found to be 5-30 $\mu\text{g mL}^{-1}$ in both medium. The regression equations were found to be: absorbance = $0.0405 \times \text{concentration in } \mu\text{g mL}^{-1} + 0.0198$ ($r^2 = 0.998$) in the pH 6.8 buffer and absorbance = $0.03904 \times \text{concentration in } \mu\text{g mL}^{-1} + 0.0598$ ($r^2 = 0.999$) in pH 1.2 buffer. The apparent molar absorptivity was found to be $1.148 \times 10^4 \text{ mol}^{-1} \text{ cm}^{-1}$ in pH 6.8 buffer and $1.105 \times 10^4 \text{ mol}^{-1} \text{ cm}^{-1}$ in pH 1.2 buffer. In both the proposed methods Sandell's sensitivity was found to be about $0.025 \mu\text{g cm}^{-2}/0.001\text{A}$. These methods were tested and validated for various parameters according to ICH guidelines and USP. The quantitation limits were found to be 1.6046 and 0.9653 $\mu\text{g mL}^{-1}$ in pH 6.8 buffer and pH 1.2 buffer, respectively. The results demonstrated that the procedure is accurate, precise and reproducible (relative standard deviation < 2 %), while being simple, cheap and less time consuming and can be suitably applied for the estimation of milnacipran hydrochloride in routine analysis.

Key Words: Milnacipran, UV-spectrophotometric methods, Validation.

INTRODUCTION

Milnacipran hydrochloride ((Z)-1-diethylaminocarbonyl-2-aminoethyl-1-phenyl-cyclopropane hydrochloride) is a cyclopropane derivative with antidepressant properties (Fig. 1). It is a nor-epinephrine serotonin reuptake inhibitor (NSRI) which is a new class of antidepressants^{1,2}. It has almost equal potency for inhibiting the reuptake of both noradrenaline and serotonin in the ratio of almost 3:1 respectively, with fewer side effects as compared to the TCA's and more efficacious than SSRI's. In January 2009, milnacipran hydrochloride was approved by US FDA for the management of fibromyalgia^{3,4}.

Recently, milnacipran hydrochloride is also approved for the treatment of chronic pain conditions like fibromyalgia and lupus for which no effective treatment is available till date. Due to all these clinical advantages of milnacipran hydrochloride over the other antidepressants and its other possible off label indications like anxiety disorders, panic disorders, neuropathic pain, obsessive compulsive disorder and stress incontinence there is increase in number of milnacipran hydrochloride formulations in the market⁴. Extensive literature survey has revealed that isocratic RP-HPLC method for the bulk and capsule formulation, Chiral RP-HPLC method for determination of milnacipran hydrochloride in tablet formu-

lation and RP-HPLC method for determination of milnacipran hydrochloride in Human plasma have been developed. But all these chromatographic techniques are not suitable for the routine analysis of milnacipran hydrochloride; hence there is a need for the development of simple, validated and cost effective UV-spectrophotometric method⁵⁻¹⁰.

The basic aim of this study was to develop and validate simple and cost effective analytical method for estimation of milnacipran hydrochloride in Bulk and Pharmaceutical formulations and *in vitro* dissolution studies of the oral formulations.

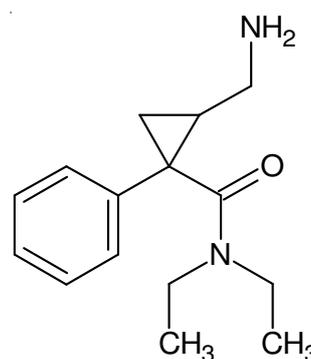


Fig. 1. Structure of milnacipran hydrochloride

TABLE-1
CALIBRATION DATA OF THE DEVELOPED METHODS (EACH VALUE IS RESULT OF NINE SEPARATE DETERMINATIONS)

Phosphate buffer medium			Hydrochloric acid medium		
Drug concentration ($\mu\text{g mL}^{-1}$)	Absorbance at 220 nm ($\pm\text{S.D.}^{\text{a}}$)	R.S.D. ^b (%)	Drug concentration ($\mu\text{g mL}^{-1}$)	Absorbance at 220 nm ($\pm\text{S.D.}^{\text{a}}$)	R.S.D. ^b (%)
5	0.2130 \pm 0.003	1.40	5	0.2454 \pm 0.002	0.99
10	0.4109 \pm 0.006	1.65	10	0.4500 \pm 0.004	0.87
15	0.6459 \pm 0.006	1.07	15	0.6582 \pm 0.004	0.54
20	0.8494 \pm 0.006	0.73	20	0.8397 \pm 0.003	0.40
25	1.0509 \pm 0.007	0.71	25	1.0464 \pm 0.005	0.49
30	1.2082 \pm 0.005	0.42	30	1.2172 \pm 0.002	0.20

^aStandard deviation. ^bRelative standard deviation

EXPERIMENTAL

Milnacipran hydrochloride (API) was obtained as gift samples from Torrent Pharmaceutical Ltd. Ahmedabad, India. Formulations containing milnacipran hydrochloride: Milnace and Milborn labeled to contain 50 mg of milnacipran hydrochloride per capsule were purchased from local Indian market. All other chemicals and reagents used were of analytical grade.

A double-beam Jasco (Japan) UV-VIS-NIR spectrophotometer, model V570 connected to computer loaded with spectra manager software. For intermediate precision study a double-beam Perkin Elmer UV-VIS spectrophotometer, model LAMBDA EZ210 connected to computer loaded with PESSW software (Version 1.2 and Revision E). Both the instruments have an automatic wavelength accuracy of 0.1 nm and matched quartz cells of 10 mm path length.

Analytical method: Various media were screened to develop a suitable UV-spectrophotometric method for the analysis of milnacipran hydrochloride in formulations. Criteria for the selection of media were sensitivity, ease of sample preparation, economy of method and its applicability. Drug solutions in the selected media were scanned in the range from 200-400 nm wavelengths for selecting the wavelength of analysis. Absorbance at the selected wavelength was determined and apparent molar absorptivity and Sandal's sensitivity were calculated.

Calibration standards: Two different stock solutions of 100 $\mu\text{g/mL}$ were prepared in the phosphate buffer (pH = 6.8) and hydrochloric acid buffer (pH = 1.2) by dissolving 10 mg of the drug in 100 mL of the respective buffer media. Different concentrations were prepared from stock solution by transferring aliquots of stock solutions into a series of 10 mL standard volumetric flasks and volumes were made with respective media. Six different concentrations were prepared in the range of 5-30 $\mu\text{g/mL}$ of milnacipran hydrochloride in the respective medium for calibration curve development. Milnacipran hydrochloride was estimated at 220 nm wavelength in both medium. The calibration data are presented in the Table-1.

Validation of analytical method: The developed method was validated in terms of specificity, selectivity, limit of detection, limit of quantification, linearity and range, precision, accuracy and robustness as per USP and ICH guidelines¹¹⁻¹³.

Specificity and selectivity: Specificity and selectivity of the proposed method was established by preparing drug solution (10 $\mu\text{g/mL}$) in both media along with and without common excipients (lactose, micro crystalline cellulose, starch,

polyvinyl pyrrolidone K-30, talc, magnesium stearate *etc.*). All samples were scanned from 200-400 nm at speed of 200 nm/min to observe any change in the absorbance at respective wavelength and spectrum.

Accuracy: Accuracy of proposed method was determined by preparing different levels of drug concentrations (LQC, MQC, HQC) in both media from the independent stock solution and analyzed (N = 9). Mean percentage recovery and percentage relative error was used for assessing the accuracy (Table-3). Accuracy of the developed method was further supported by performing standard addition method. In this method different concentration of pure drug (5, 12, 20 $\mu\text{g/mL}$ in the phosphate buffer and hydrochloric acid buffer) were added to known pre analyzed formulation sample and total concentration was determined using the proposed methods (N = 3). The amount of added concentration was determined by calculating percent recovery $\% \text{ recovery} = [(C_v - C_u)/C_a] \times 100$, where C_v is total drug concentration measured after standard addition. C_u is drug concentration in formulation, C_a is drug concentration added to formulation (Table-4).

Precision: Repeatability was determined by using different level of drug concentration (same concentration as in accuracy study) prepared from the independent stock solution and analyzed (N = 9). Intraday precision was determined by analyzing three different concentration of drug in triplicate for three times in same day (N = 27). Interday precision was performed by following same procedure at three different days. Instrument variation was determined by reanalyzing three different concentration (LQC, MQC and HQC) samples using Perkin-Elmer instrument by same method. The relative standard deviation (in %) of the predicted concentrations from the regression equation was taken as precision (Table-5).

Linearity: Linearity of the proposed method was established by plotting standard calibration curve over the concentration range of 5-30 $\mu\text{g/mL}$ in both media (N = 9). Least square regression analysis was done for the obtained data.

Limit of detection (LOD) and limit of quantitation (LOQ): LOD and LOQ were determined by using calibration curve data. Limit of detection was determined by using formula $3.3 \sigma/S$ and LOQ was determined by using formula $10 \sigma/S$ (where σ is standard deviation of intercept and S is the slope of calibration curve).

Robustness: Three different concentrations (LQC, MQC and HQC) are taken for assessment of robustness of proposed method by changing the pH of media by ± 0.2 unit and calculating mean % recovery in both the media.

TABLE-2
OPTICAL CHARACTERISTICS, STATISTICAL DATA OF THE REGRESSION EQUATIONS AND VALIDATION
PARAMETERS (EACH VALUE IS RESULT OF NINE SEPARATE DETERMINATIONS)

Parameter	Phosphate Buffer (pH 6.8)	Hydrochloric acid buffer (pH 1.2)
<i>Optical characteristics</i>		
Apparent molar absorptivity ($1 \text{ mol}^{-1} \text{ cm}^{-1}$)	1.148×10^4	1.105×10^4
Sandell's sensitivity ($\mu\text{gcm}^{-2}/0.001\text{A}$)	0.0246	0.0256
<i>Regression analysis</i>		
Slope (S.E. ^a)	0.0405 (3.0×10^{-4})	0.03904 (2.87×10^{-4})
95% confidence limits of slope	0.03765; 0.04335	0.03732; 0.04073
Intercept (S.E. ^a)	0.0198 (4.6×10^{-3})	0.0598 (6.2×10^{-3})
95% confidence limits of intercept	-0.03568; 0.07517	0.02666; 0.09303
Standard error of estimate	1.206×10^{-3}	1.468×10^{-3}
Regression coefficient (r^2)	0.998	0.999
<i>Validation parameters</i>		
Linearity ($\mu\text{g mL}^{-1}$)	5-30 $\mu\text{g mL}^{-1}$	5-30 $\mu\text{g mL}^{-1}$
Limit of detection ($\mu\text{g mL}^{-1}$)	0.5294 $\mu\text{g/mL}$	0.3186 $\mu\text{g/mL}$
Limit of quantification ($\mu\text{g mL}^{-1}$)	1.6043 $\mu\text{g/mL}$	0.9653 $\mu\text{g/mL}$
Robustness (mean % recovery \pm S.D.)	99.16 ± 0.0014	100.3 ± 0.0067

^aStandard error of mean

Estimation from formulation: Five capsules were weighed and crushed the content. Amount of powder equivalent to 25 mg of milnacipran hydrochloride was weighed and extracted with phosphate buffer medium and hydrochloric acid medium separately for 0.5 h by sonicating the solution. The solutions were diluted suitably to prepare 20 $\mu\text{g/mL}$ concentrations in respective media. Finally, solutions were filtered through whatman filter paper number 40 and filtrates were analyzed at 220 nm for both media respectively.

RESULTS AND DISCUSSION

The selection of the analytical media is crucial for estimation of milnacipran hydrochloride as milnacipran hydrochloride shows the pH dependent solubility. The pH of final media decided was 100 mM phosphate buffer (pH 6.8) and 100 mM hydrochloric acid buffer (pH 1.2) based on sensitivity of method, cost and applicability of method to dissolution studies and ease of preparation. The λ_{max} of milnacipran hydrochloride was 220 nm in both phosphate buffer medium and hydrochloric acid buffer medium. Apparent molar absorptivity of drug was found to be 1.105×10^4 and 1.148×10^4 in hydrochloric acid buffer medium and phosphate buffer medium respectively. Sandell's sensitivity of drug was found to be 0.0256 and 0.0246 in hydrochloric acid buffer medium and phosphate buffer medium respectively as shown in Table-2.

Calibration curve: The linear regression equation obtained at 220 nm was: absorbance = $[0.0405 \times \text{conc. in } \mu\text{g mL}^{-1}] + 0.0198$ with regression coefficient of 0.997 and absorbance at 220 nm = $[0.0390 \times \text{conc. in } \mu\text{g mL}^{-1}] + 0.0598$ with regression coefficient of 0.999 in phosphate buffer and hydrochloric acid buffer medium respectively.

Specificity and selectivity: The UV spectrum of milnacipran hydrochloride was same as absorbance spectrum of pure drug alone and in presence of common excipients in both medium (Fig. 2). Hence, proposed methods are specific and selective for drug.

Accuracy: Accuracy in phosphate buffer was found to be in range of -1.37 % to 1 % and -0.63 % to 0.89 % in phosphate buffer medium and hydrochloric acid buffer medium respectively.

The mean % recovery values are nearly 100 % with low SD values (<1.5) shows accuracy of method. The accuracy of proposed method was further validated by performance recovery studies of standard addition method (Tables 3 and 4). The mean recoveries (% RSD) for lower, intermediate and higher conc. were found to be 100.04 (0.211), 99.86 (0.225) and 100.04 (0.167) respectively in phosphate buffer medium. Mean % recovery (% RSD) for lower, intermediate and higher conc. were found to be 100.10 (0.324), 100.02 (0.174) and 100.03 (0.093) respectively in hydrochloric acid buffer medium. The result shows that the proposed method can accurately measure any small change in drug conc. in the solution.

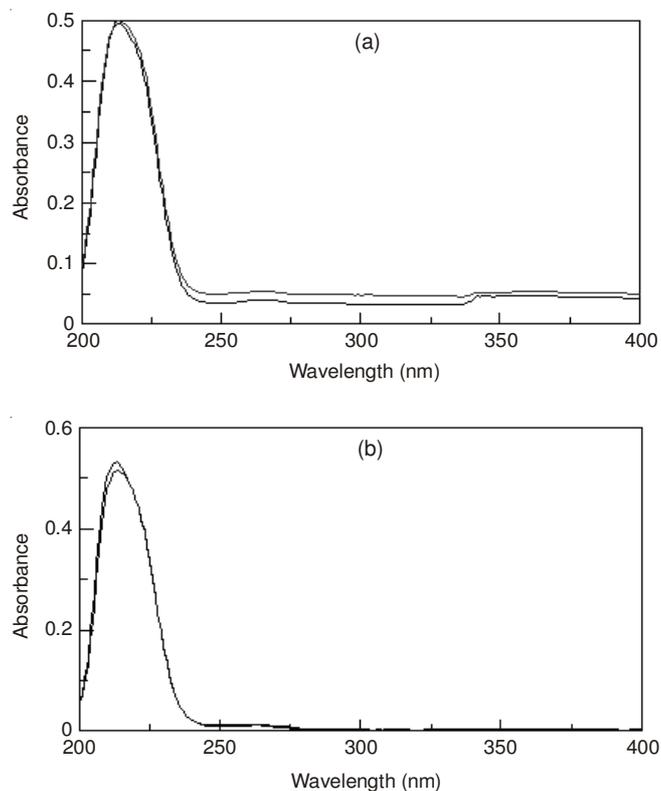


Fig. 2. (a) spectra in pH 6.8 buffer, (b) spectra in pH 1.2 buffer

TABLE-3
ACCURACY AND PRECISION DATA FOR THE DEVELOPED METHODS
(EACH VALUE IS RESULT OF NINE SEPARATE DETERMINATIONS)

Level	Predicted conc. ($\mu\text{g mL}^{-1}$) ^a			Mean % recovery (\pm S.D.)	Accuracy (% relative error) ^b
	Range	Mean (\pm S.D.)	R.S.D (%)		
Phosphate buffer					
LQC	5.96 - 6.17	6.06 \pm 0.07	1.21	101.03 \pm 1.296	1.0
MQC	17.98 - 18.19	18.07 \pm 0.06	0.32	100.39 \pm 0.345	0.39
HQC	26.47- 26.80	26.63 \pm 0.08	0.29	98.62 \pm 0.311	-1.37
Hydrochloric acid buffer					
LQC	5.82 - 6.09	5.99 \pm 0.089	1.50	99.94 \pm 1.499	-0.17
MQC	17.96 - 18.27	18.16 \pm 0.11	0.59	100.87 \pm 0.600	0.89
HQC	26.64 - 26.98	26.83 \pm 0.10	0.38	99.38 \pm 0.377	-0.63

LQC = 6 $\mu\text{g mL}^{-1}$, MQC = 18 $\mu\text{g mL}^{-1}$, HQC = 27 $\mu\text{g mL}^{-1}$; ^aPredicted concentration of milnacipran hydrochloride was calculated by linear regression equation; ^bAccuracy is given in % relative error (= 100 \times [(predicted concentration-nominal concentration)/nominal concentration])

TABLE-4
RESULTS OF STANDARD ADDITION METHOD (EACH VALUE IS RESULT OF THREE SEPARATE DETERMINATIONS)

Method	Concentration of drug formulations ($\mu\text{g mL}^{-1}$)	Concentration of pure drug added ($\mu\text{g mL}^{-1}$)	Total concentration of drug found ($\mu\text{g mL}^{-1}$)	Analytical recovery (%) (\pm S.D.)
Phosphate Buffer medium	10.18	5	15.19	100.04 \pm 0.211
	10.18	12	22.15	99.86 \pm 0.225
	10.18	20	30.19	100.04 \pm 0.167
Hydrochloric acid buffer	8.20	7	15.22	100.10 \pm 0.324
	8.20	15	23.21	100.02 \pm 0.179
	8.20	24	32.21	100.03 \pm 0.093

TABLE-5
RESULTS OF INTERMEDIATE PRECISION STUDY

Concentration ($\mu\text{g mL}^{-1}$)	Intra-day repeatability % R.S.D. (N = 9)			Inter-day repeatability % R.S.D. (N = 27)	Inter-instrument repeatability % R.S.D. (N = 6)
	Day 1	Day 2	Day 3		
Phosphate buffer medium					
LQC	1.11	1.17	0.87	1.33	1.45
MQC	0.77	0.46	0.37	0.59	1.13
HQC	0.83	1.02	0.75	0.84	1.25
Hydrochloric acid medium					
LQC	1.33	0.98	1.30	1.35	1.41
MQC	0.84	0.84	0.52	0.72	1.03
HQC	0.79	0.58	0.43	0.64	0.59

Precision: Precision of the proposed methods was studied by evaluating repeatability and intermediate precision. Repeatability (% RSD) ranged from 0.37 % to 1.17 % and 0.43 % to 1.33 % in phosphate buffer medium and hydrochloric acid buffer medium respectively, at all three levels of concentrations (Table-5). This shows the precision under same operating conditions over a short interval of time in a day. Intermediate precision shows variations in different day. In intermediate precision study excellent % RSD values were found out (< 1.5 %) in all the cases.

This % RSD values shows that these methods have good repeatability and intermediate precision.

Linearity: The linearity range was found to be 5-30 $\mu\text{g/mL}$ in both the selected media at 220 nm. Lower values of parameters like standard error of slope and intercept (Table-2) indicated high precision of the proposed method. The mean slope and intercept values are within the 95 % confidence interval.

LOD and LOQ: LOD and LOQ in phosphate buffer medium was found to be 0.5294 $\mu\text{g/mL}$ and 1.6043 $\mu\text{g/mL}$ respectively and in hydrochloric acid buffer medium LOD and

LOQ were found to be 0.3185 $\mu\text{g/mL}$ and 0.9653 $\mu\text{g/mL}$ respectively.

Robustness: Variation of pH of the selected media by \pm 0.2 did not have any significant effect on absorbance. The mean % recovery (\pm S.D.) was found to be 100.94 \pm 0.54 and 100.13 \pm 0.50 in the phosphate buffer and hydrochloric acid media, respectively (Table-6).

TABLE-6
RESULTS OF ROBUSTNESS STUDY

Concentration ($\mu\text{g mL}^{-1}$)	Mean % Recovery ^a \pm SD (N = 9)	
	Phosphate buffer medium pH 7	Hydrochloric acid medium pH 1.4
LQC	100.20 \pm 1.48	99.15 \pm 0.99
MQC	100.94 \pm 0.54	101.92 \pm 0.71
HQC	98.34 \pm 0.31	100.13 \pm 0.50

^aEach value is mean of three separate determinations

Estimation from formulation: In phosphate buffer the assay values of milnacipran hydrochloride for different formulations ranged from 99.41 to 100.39 % with standard deviation not more than 1.17 %. In hydrochloric acid medium the assay

TABLE-7
APPLICATION OF THE PROPOSED SPECTROPHOTOMETRIC METHODS TO THE DETERMINATION OF MILNACIPRAM HYDROCHLORIDE IN DOSAGE FORMS (EACH VALUE IS THE AVERAGE OF FIVE SEPARATE DETERMINATIONS)

Commercial products	Phosphate buffer medium		Hydrochloric acid medium	
	Amount found	Assay (%)	Amount found	Assay (%)
Milnace capsule 50 mg (Torrent pharmaceutical Ltd. India)	49.70 ± 0.50	99.41 ± 1.0	49.89 ± 0.16	99.77 ± 0.32
Milborn capsule 50 mg (SUN Pharma. India)	50.20 ± 0.59	100.39 ± 1.17	50.42 ± 0.54	100.85 ± 1.07

values of milnacipran hydrochloride for different formulations ranged from 99.77 to 100.85 % with standard deviation not more than 1.07 % (Table-7).

Conclusion

The proposed methods are quite simple and do not require any pretreatment of the drug and tedious extraction procedure. The methods have wider linear range with good accuracy and precision. Hence, the data presented in the manuscript by spectrophotometric methods for the determination of milnacipran hydrochloride in its pure and dosage form demonstrate that the proposed methods are accurate, precise, linear, selective and offer advantages of reagent availability and stability, less time consumption and high sensitivity. Thus it can be extended for routine analysis of milnacipran hydrochloride in pharmaceutical industries, hospitals and research laboratories. Unlike the gas chromatography and high performance liquid chromatography procedures, the UV-visible spectrophotometer instrument is simple and not of high cost. Moreover the methods are free from interferences by common additives and excipients.

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