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Research Article

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Method development and validation for determination and quantitative estimation of impurities in milnacipran hydrochloride by liquid chromatography technique

Kanchan Nautiyal and K. Ramakrishna

Department of Chemistry, Institute of Science, GITAM University, Visakhapatnam, Andhra Pradesh, India

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ABSTRACT

The present paper describes a simple gradient reverse phase Chromatographic method for the quantification of organic impurities in Milnacipran (MCP). Detection ,estimation and good quality resolution was achieved between Milnacipran and Nine impurities using C_{18} (100mm×4.6mm,2.7µ) column using a gradient elution pattern with buffer of Octane -1-sulphonic acid and solvent as Acetonitrile and Methanol at a flow rate was 1.0 ml/min and the detection was carried out at 210nm. The factors involved in the method development were discussed. The method was validated as per International Conference on Harmonization (ICH) guidelines in terms of specificity, system suitability, precision, accuracy, linearity and was able to quantitate all the nine impurities. The method was able to quantitate up to 0.404 ppm of Imp-1, 0.398 ppm of Imp-2, 0.474 ppm of Imp-3, 0.407 ppm of Imp-4, 0.400 ppm of Imp-5, 0.402 ppm of Imp-6, 0.401 ppm of Imp-7, 0.398 ppm of Imp-8, 0.404 ppm of Imp-9 and 0.407 ppm of Milnacipran.

Keywords: Development, Validation, Milnacipran, impurities, HPLC, Column

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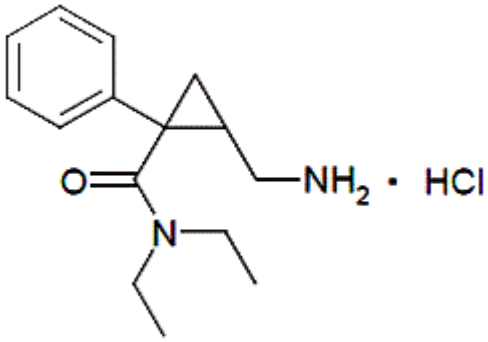
INTRODUCTION

Milnacipran, is a selective norepinephrine and serotonin reuptake inhibitor and inhibits norepinephrine uptake with greater potency than serotonin .It is a racemic mixture with the chemical name (±)-[1R(S),2S(R)]-2-(amino methyl)-N,N-diethyl-11phenylcyclopropane carboxamide hydrochloride (Figure-1).Milnacipran can be used for the treatment of fibromyalgia.It's usually administered orally in the form of tablets. A few literature was reported by non-conventional techniques like colorimetric [1] and by HPTLC [2-3] in dosage forms. Most of the reported HPLC method was related to determination of milnacipran in dosage forms [4-12] and few other chromatographic methods were reported using special detection technique of LCMS/MS [13-14] for estimation in plasma samples. A couple Method for the identification and quantification of impurities in Milnacipran API was reported but both the literature used U-HPLC [15-16] and the impurity estimated in these methods was either single or two. However there were no reports available on related substances method for Milnacipran API for more than two impurities and dosage forms. So sincere effort was made to develop a simple and easy Reverse HPLC method for estimation of related substances in Milnacipran API and validated as per ICH guidelines to prove the developed method was accurate and precise.

Figure 1: Structure of Milnacipran Hydrochloride



EXPERIMENTAL SECTION

Required Equipment's like HPLC (Agilent and shimadzu) equipped with auto sampler and photodiode array detector. Column C_{18} (100x4.6mm, 2.7µ), Millipore filtration kit, mobile phase reservoir, sample filtration assembly and glass wares were used throughout the experiment.

Chemicals and Solvents

Milnacipran and impurities, viz. Imp-1, Imp-2, Imp-3, Imp-4, Imp-5, Imp -6, Imp-7 ,Imp-8 and Imp-9 were obtained from ShankoBiochem.Octane-1-sulphonic acid, methanol, Acetonitrile, Triethylamine,Sulphuric acid were obtained from Merck.

Chromatographic Parameters

Equipment: HPLC equipped with injector, Pump, UV/PDA detector and recorder
Column: C_{18} (100 x 4.6, 2.7µ)
Flow rate: 1.0 ml/min
Wavelength: 210 nm
Injection Volume: 10 µL
Column Temperature: 40°C
Run time: 75 mins.
Auto sampler Temp: 10°C

Gradient Program:

Time	Mobile Phase A(%)	Mobile Phase B(%)
0.00	85	15
2.00	85	15
18.00	80	20
50.00	48	52
52.00	48	52
63.00	25	75
65.00	85	15

Preparation of Mobile phase

Buffer Preparation

Weighed about 1.0 g of Octane-1-sulphonic acid sodium salt in 2000 ml of water, sonicate, and filtered through micron filter.

Mobile Phase Preparations

Mobile Phase-A

Mixed buffer solution and Acetonitrile in the ratio of 90:10, adjusted pH of the mixture to 2.3 with Sulphuricacid, sonicated to degas.

Mobile Phase-B

Mixed buffer solution, Acetonitrile and Methanol in the ratio of 25:60:15, sonicated to degas.

Diluent

Mixed Buffer solution and acetonitrile in the ratio of 90:10, adjusted pH of the mixture to 7.0 with dilute ammonia, sonicated to degas.

Preparation of Solutions

Stock Solution Preparation

Weighed about 5 mg of Imp-6 standard in a 100 ml volumetric flask, add 5 ml of acetonitrile, sonicated and dilute to volume with diluent.

System suitability Preparation

Weighed accurately about 25 mg of Milnacipran Hydrochloride standard in a 25 ml volumetric flask, add 10 ml of diluent,sonicated,1ml of stock solution added, diluted to the volume with diluent.

Standard Solution Preparation

Weighed about 75 mg Milnacipran standard in 100 ml volumetric flask dissolved and dilute to 100 ml with diluent. Further diluted 5.0 ml of this solution in 50 ml volumetric flask and diluted to volume with diluent, further diluted 1.0 ml of this solution in 50 ml volumetric flask and diluted to 50 ml with diluent.

Preparation of Test solution

Weighed accurately about 50 mg of Milnacipran sample in 50 ml of volumetric flask dissolve and diluted to 50 ml with diluent.

METHOD VALIDATION

The proposed method was validated as per ICH guidelines. The preparations were adopted as mentioned in experiment section.

System suitability and specificity

System suitability was performed by injecting standard solution preparation in six times, and measured the system suitability parameters like theoretical plates, tailing factor and % RSD were evaluated, non-interference of blank and impurity peaks with the active peak and should be separated each other was proved by analyzing spiked solution with all impurities and all the results were well within the criteria and results compiled in Table-1, 2 and Figure-2, 3, 4 and 5.

Table 1: Results of System Suitability study

System Suitability		
Parameter	*Observation*	*Acceptance criteria*
Tailing factor for MCP Peak	1.23	NMT 2.0%
% Relative standard deviation for six replicate injections	1.34	NMT 5.0%
Resolution between Imp-6 and Milnacipran Hcl	6.82	NLT 2.0

Figure 2: Typical Chromatogram of Blank solution

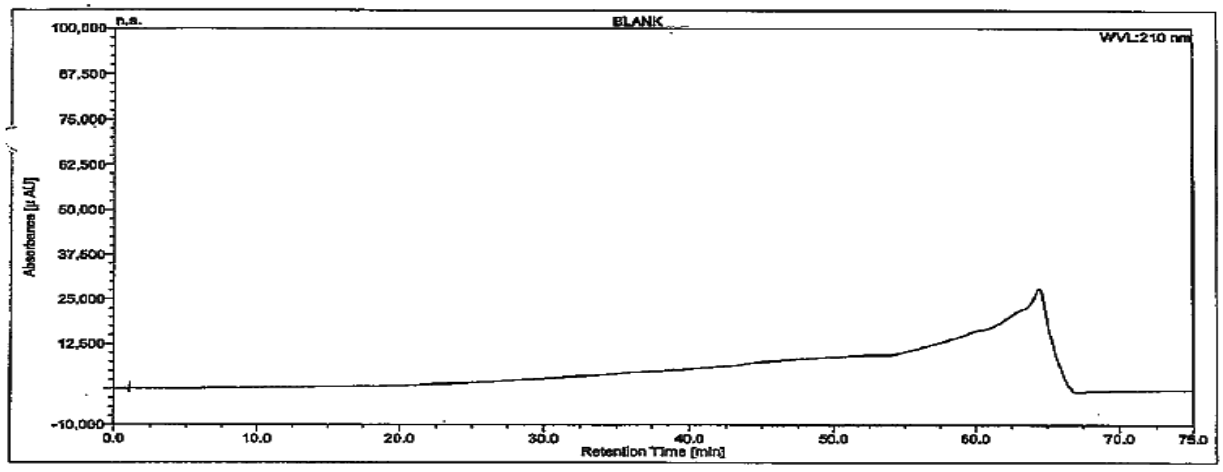


Table 2: Results of Specificity and forced degradation study data

Analyte	Degradation Type					
Impurity	Acid	Alkali	Oxidative	Thermal	Humidity	Photolytic
Impurity-2	1.20	4.3	0.21	ND	ND	ND
Impurity-3	ND	0.17	ND	ND	ND	ND
Impurity-4	ND	ND	0.10	ND	ND	ND
Impurity-5	ND	ND	0.65	ND	ND	ND
Unknown at RRT 0.13	ND	ND	0.15	ND	ND	ND
Unknown at RRT 0.25	ND	1.28	ND	ND	ND	ND
Unknown at RRT 0.57	ND	ND	0.90	ND	ND	ND
Unknown at RRT 0.64	ND	ND	0.17	ND	ND	ND
Unknown at RRT 0.74	ND	0.60	ND	ND	ND	ND
Total Imp	1.18	6.34	3.03	NA	NA	NA

Figure 3: Typical Chromatogram of Specificity (Spiked) solution

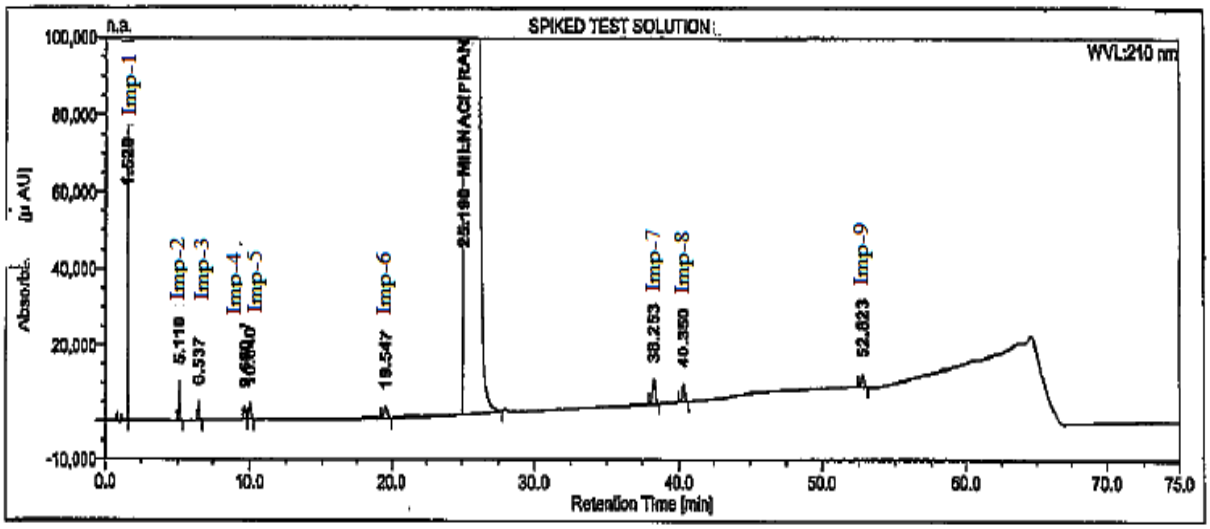


Figure 4: Typical chromatogram of System suitability

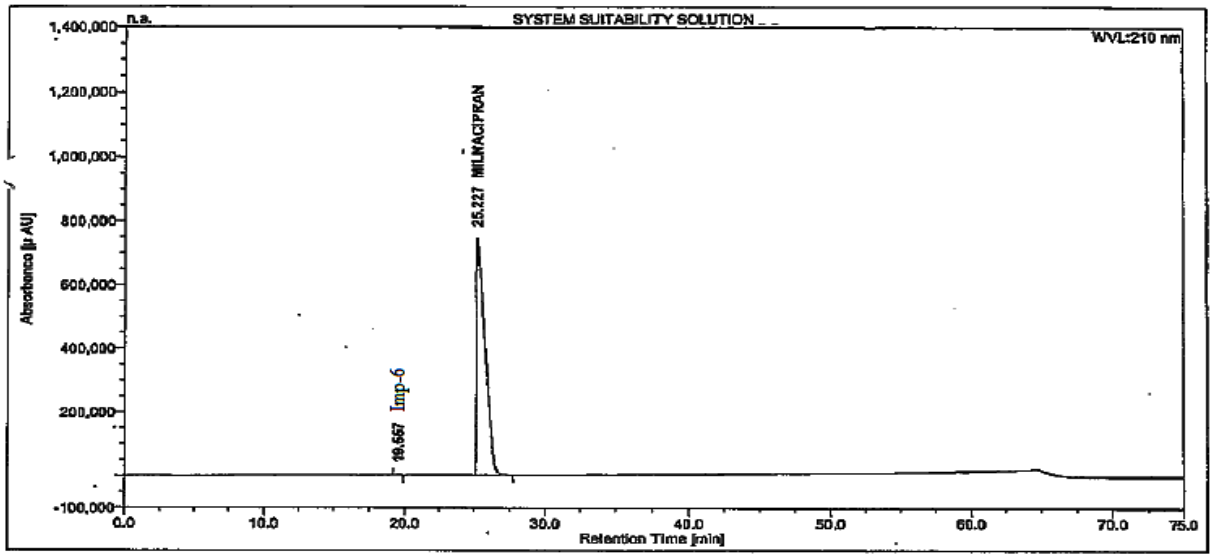
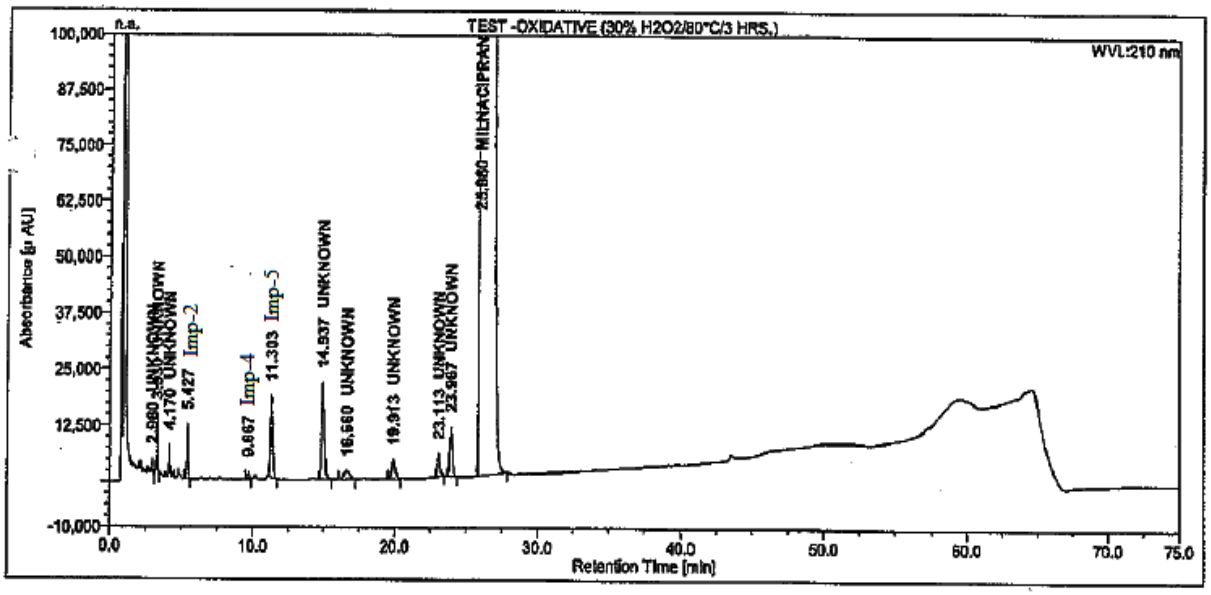


Figure 5: Typical chromatogram of Forced Degradation



Limit of Detection and Quantification

To determine the limit of detection and quantification, analyzed an appropriate number of diluted solutions of impurities and active, linearity graph was drawn, limit of detection and quantification was calculated from graph and levels in ppm are mentioned in Table-3 and Figure-6 & 7.

Table 3: Results of Limit of Detection and Quantification Study

Peak Name	LOD		LOQ	
	Concentration	ppm	Concentration	ppm
Imp-1	0.001	0.003	0.001	0.008
Imp-2	0.00	0.003	0.001	0.008
Imp-3	0.001	0.006	0.002	0.018
Imp-4	0.001	0.012	0.004	0.038
Imp-5	0.001	0.001	0.002	0.017
Imp-6	0.001	0.009	0.003	0.027
Imp-7	0.001	0.003	0.001	0.01
Imp-8	0.002	0.017	0.005	0.052
Imp-9	0.001	0.014	0.004	0.042
Milnacipran	0.001	0.01	0.003	0.032

Figure 6: Typical Chromatogram of Limit of Detection

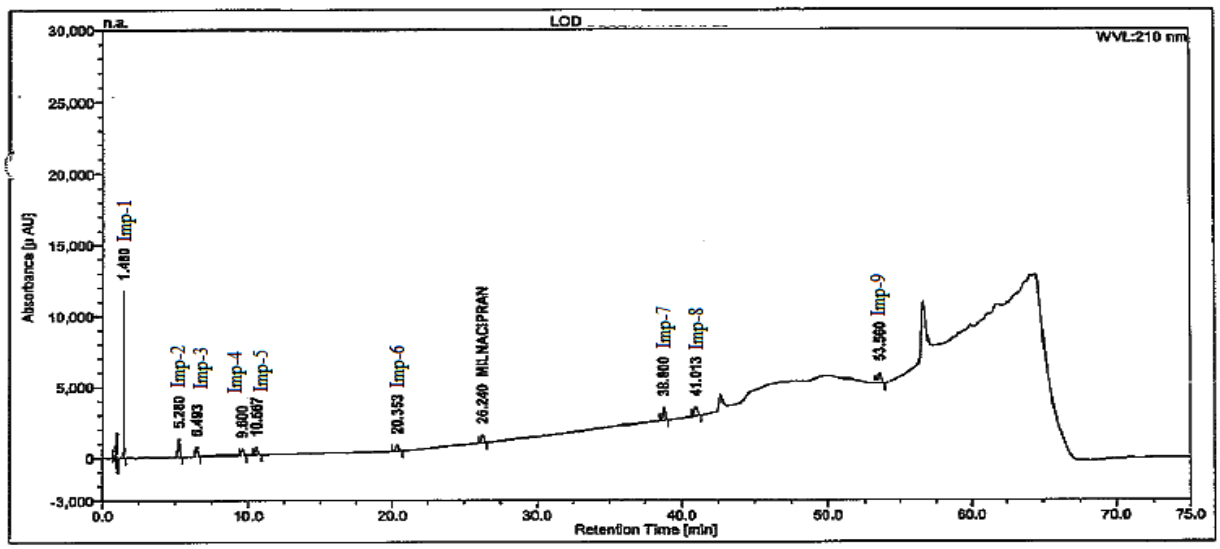
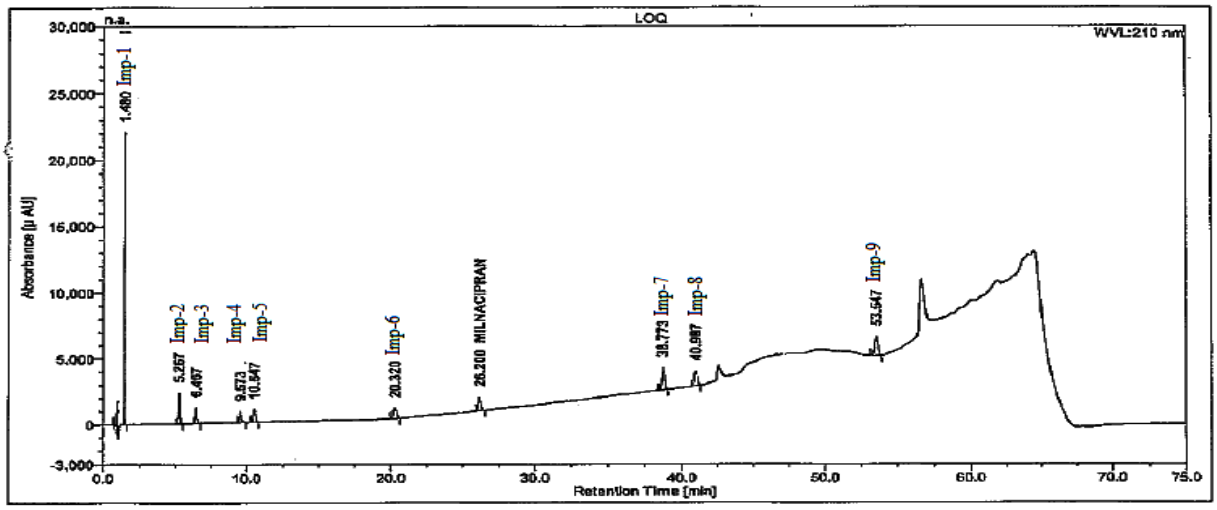


Figure 7: Typical Chromatogram of Limit of Quantification



Linearity

Linearity study was performed by preparing spiked solution of impurities and Milnacipran over the range of LOQ to 150% of specification level concentration. The area response was calculated and linearity graph derived and its slope, intercept and correlation coefficient was calculated and well within the acceptance criteria of 0.997 which indicates the method is linear in nature and results were compiled in Table-4 and representative chromatograms and graphs were highlighted Figure-8 & 9.

Figure 8: Typical chromatogram of Linearity

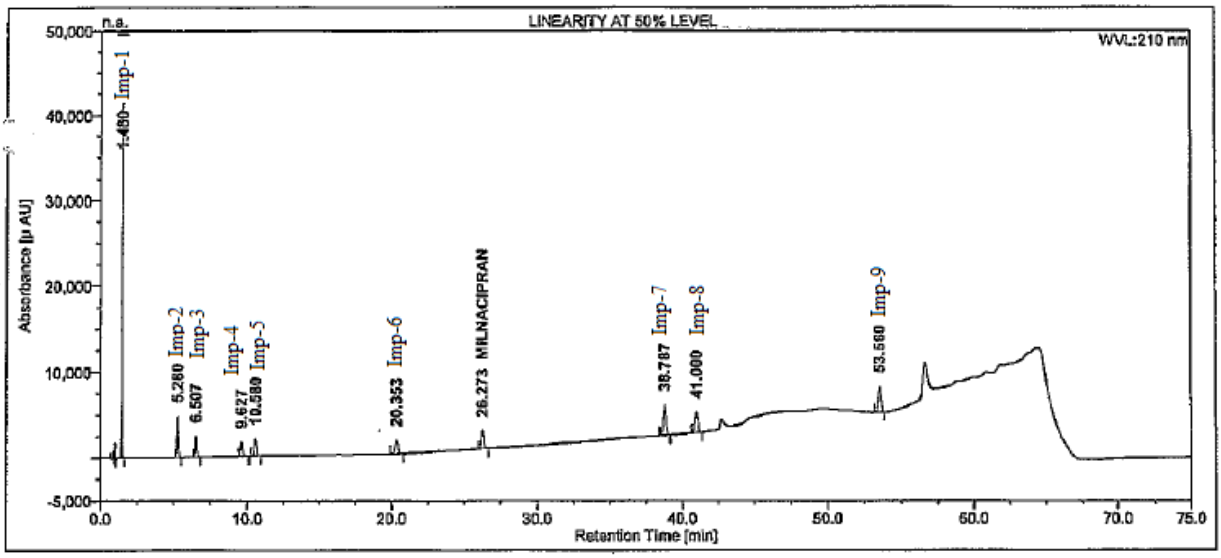
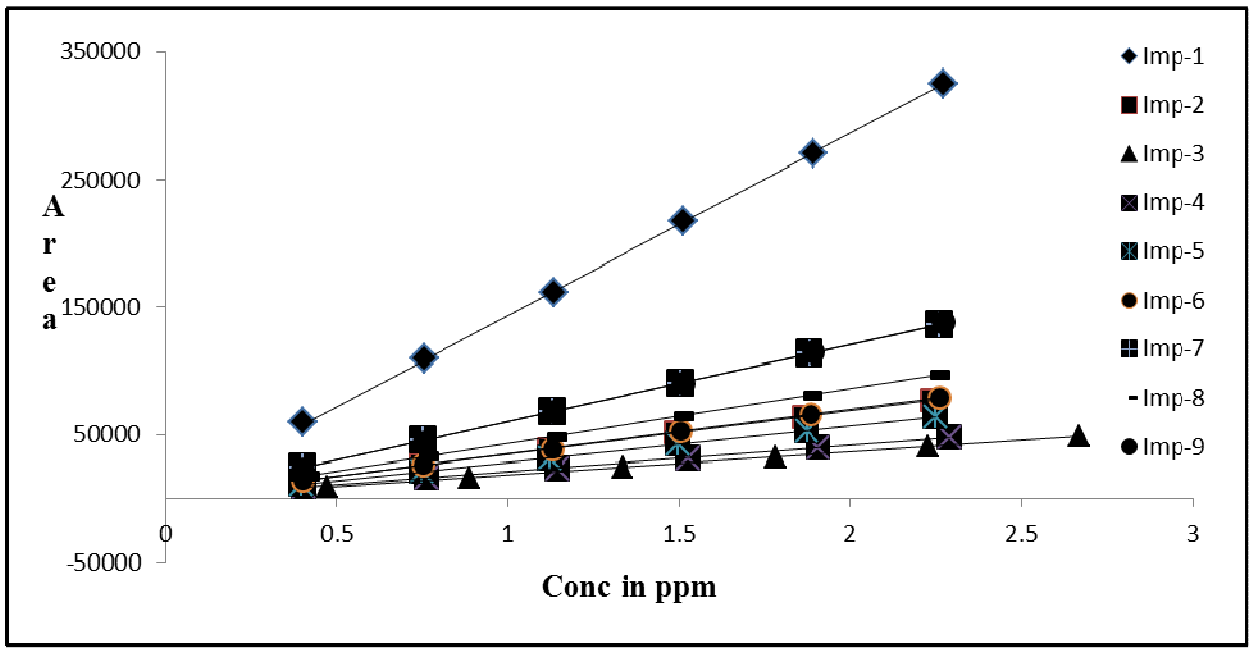


Table 4: Results of Linearity and Range Study

Results	Concentration (ppm)	Slope	Intercept	Correlation Coefficient	Residual sum of squares
Imp-1	0.404-2.271	141957	1887	0.99995	4808097
Imp-2	0.398-2.236	34358	-3.74	0.99998	139165
Imp-3	0.474-2.669	18400	-173	0.99994	128921
Imp-4	0.407-2.289	20675	-57	0.99998	38552
Imp-5	0.400-2.248	28180	12	0.99999	49907
Imp-6	0.402-2.260	34979	-239	0.99996	261456
Imp-7	0.401-2.258	60141	68	0.99999	202980
Imp-8	0.398-2.236	43055	-246	0.99995	426400
Imp-9	0.404-2.270	62128	-295	0.99999	229266
Milnacipran	0.407-2.289	32188	-333	0.99993	379020

Figure 9: Linearity Curve of Milnacipran and its impurities



Precision

System Precision

System precision was performed by injecting standard solution preparation in six replicates, the area response and % RSD were calculated and results were compiled and found well within the acceptance criteria.

Method precision

This experiment was performed by injecting six test preparations as per methodology of single batch and determined the organic impurities of the same, percentage and relative standard deviation of organic impurities of the results were calculated and results were compiled and results indicates the precision of the developed method.

Intermediate Precision (Ruggedness)

To demonstrate the reproducibility of the method, performed by injecting six test preparations for variability of instrument, column, day and analyst and determined the organic impurities of the same, percentage and relative standard deviation of the results of organic impurities were calculated and results were compiled and results indicates the ruggedness of the method.

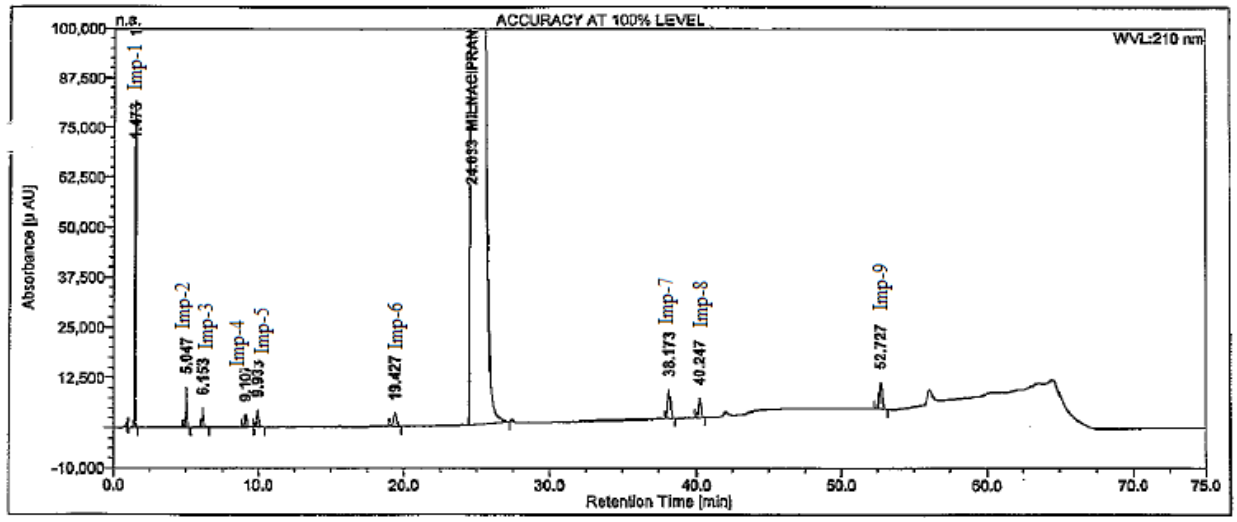
Accuracy Study (Recovery Study)

Accuracy of the analytical method was performed by spiking known quantities of all impurities (at LOQ ,100% and 150% level of specification level concentration) to test sample in triplicate preparations and recovery was calculated and well within the acceptance criteria of 85% to 115% and shows the method is accurate and precise. The results of recovery study were compiled in Table-5 and Figure-10.

Table 5: Results of Accuracy study.

Level	Imp-1	Imp-2	Imp-3	Imp-4	Imp-5	Imp-6	Imp-7	Imp-8	Imp-9
	% Recovery								
LOQ	103.39	99.41	99.30	101.55	93.58	101.0	91.02	99.58	103.05
100%	101.45	101.56	101.37	101.59	103.43	101.95	98.63	101.36	101.9
150%	101.38	101.54	101.91	101.75	102.69	101.92	99.03	101.45	101.09

Figure 10: Typical chromatogram of Accuracy study



Stability of Analytical solution

Standard, Test and spiked test solution was prepared as per methodology and stored at 10°C, these solutions injected at regular intervals for 48 hours, % difference of analyte peak area for standard and test solutions with that of initial and all were within the limit of ±2 and solution stability was established up to 12 hours.

Robustness

The Robustness of analytical method was established by demonstrating its reliability against deliberate changes in chromatographic condition and impact on system suitability parameters were monitored and the values are well within the acceptance criteria.

RESULTS AND DISCUSSION

The mobile phase consisting of mixture of buffer: Methanol : Acetonitrile of various ratio with gradient program of elution, at 1ml/min flow rate was optimized to which gave well Seperation of all impurities, uv absorption pattern shows the all the nine impurities and Milnacipran were absorbed appreciably at 210 nm, so this wavelength was selected as a detection wavelength for analysis, to maintain the sharpness of impurities peak ,the column temperature was maintained 40°C ,as analytical solution has stability variation on storage, so sampler temperature condition was kept as 10°C.The retention times for Milnacipran and nine impurities were 25.1 min,1.52 min,5.10 min,6.50 min,9.60 min,10.0 min,19.50 min,38.2 min,40.3 min and 52.8 min respectively. The proposed method was successfully applied to identification and quantification of impurities in Milnacipran.

The method validation was performed, specificity results was reflecting there was no interference of blank and its impurities with Milnacipran. The LOD for impurities were in the range of 0.003 to 0.017 ppm and LOQ for impurities compound were in the range of 0.40 to 0.480 ppm and linearity and range was in the range of 0.40 to 2.70 ppm.The robustness study results was included as method precision which has results of 0.20 to 0.60% RSD and intermediate precision result were 0.05 to 0.75 % RSD.The recovery of all impurities proposed method was studied and results were in the range 90.5-104%.In the proposed study,a simple analytical method was developed for quantifying all the nine impurities in Milnacipran and validated as per ICH guide lines. Statistical analysis proved that method was accurate, precise and reproducible.

CONCLUSION

The reverse phase HPLC method was optimized and established for appropriate shapes and resolutions of Milnacipran and its Nine impurities and method was validated, based on validation results and interpretation of values, it was concluded that this RP HPLC was simple, accurate and precise and method presented in this paper can be successfully used for routine monitoring and quantification of Impurities in Milnacipran Hydrochloride..

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