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Research Article

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Development and Validation of Novel RP-HPLC Techniques for the Quantification of Contaminants

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Abstract According to ICH Guidelines, the suggested approach was validated. The findings of the specificity investigation were noted and showed that there was no interaction between Milnacipran and blank or its contaminants. The findings showed that the Limit of Quantification (LOQ) and Limit of Detection (LOD) ranged from 0.40 to 0.480 ppm and 0.003 to 0.017 ppm, respectively. The fact that the suggested technique produced the lowest LOD and LOQ values suggests how sensitive the devised method is. There was a strong linear relationship (correlation coefficient: 0.9990) found between the concentration and matching impurity and Milnacipran peak regions. The linearity ranged from 0.40 to 2.70 ppm. By examining the test solution laced with contaminants at different known concentrations, the accuracy (recovery) of all the impurities of the suggested approach was examined. By comparing the area of the Milnacipran sample before and after the addition of contaminants, the recovery was computed, and the findings ranged from 91.02% to 103.43%. The numbers were discovered to be within the acceptable range, demonstrating the current method's accuracy.

Keywords ICH Guidelines, Quantification, Milnacipran, Accuracy

Introduction

The Greek word "pharmakeutikos" is where the term "pharmaceutical" comes from [1]. The pharmaceutical sector grows and produces pharmaceuticals [2]. German dye manufacturers were effective in documenting organic separation's foundational technology [3]. The development of artificial organic technologies allowed scientists to carefully manipulate the configuration of chemicals, and advancements in the field of pharmacology extended their capacity to evaluate the biotic characteristics of these configuration changes [4]. Hermann Emil Fischer reported the discovery of diethylbarbituic acid in the early 1800s. It is a compound formed of diethylmalonic acid, phosphorus oxychloride, and urea. This aids in inducing the inclination to sleep in animals, particularly dogs. It was discovered on proprietary property and approved by Bayer Pharmaceuticals, which began selling the substance under the brand name Veronal as a sleeping aid at the beginning of 1904 [5].

Following systematic studies on the effects of arrangement changes on the efficacy and scope of the act, Phenobarbital [6] was discovered by Bayer between 1911 and 1912; this led to the discovery of its anti-epileptic properties. Until 1970 [7], phenobarbital was one of the most widely prescribed drugs for the treatment of epilepsy [8-9]. It is still included as a necessary prescription on the WHO's essential pharmaceuticals list [10]. Increased awareness of addiction throughout the 1950s and 1960s led to use restrictions and a rise in prescriber regulatory violations [11].

Research on cancer treatments began in 1970, and starting in 1978, India emerged as a major hub for pharmaceutical manufacture free from patent restrictions [12]. Following 2008, pharmaceutical companies raised the prices of their brand-name prescription pharmaceuticals on a regular basis to offset declining earnings as a result of the sharp rise of generics on the market [13].

For the purpose of finding and creating novel medications, drug discovery is essential. Historically, a greater proportion of medications have been created by accidental discovery or by isolating the active component from conventional treatments [14]. In recent times, there has been a focus on acknowledging the metabolic pathways associated with a disease or pathogen and using molecular biology or biochemistry to influence these pathways. Research on novel drugs has been traditionally mandated by educational and scientific establishments [15].

The primary goals of drug advancement are to identify appropriate formulation preparation and medicating mode as well as to determine medication safety. Clinical studies are often conducted after an amalgamation of in vitro and in vivo data in these domains of study. The amount of capital required for late-stage development has made it a competitive advantage for larger pharmaceutical companies.

Materials and Methods

Essential equipment such as Shimadzu and Agilent liquid chromatography systems equipped with auto samplers and photodiode array detectors. Throughout the experiment, glassware, a Millipore filtration kit, a mobile phase reservoir, a sample filtration assembly, and Column C18 (100x4.6mm, 2.7µ) were used.

Chemicals and Solvents

ShankoBiochem provided milnacipran and its impurities, which were Impurity-1, Impurity-2, Impurity-3, Impurity-4, Impurity-5, Impurity-6, Impurity-7, Impurity-8, and Impurity-9. Analytical-grade octane-1-sulphonic acid, methanol, acetonitrile, triethylamine, and sulfuric acid were acquired from Merck.

Preparation of Mobile phase

Buffer Preparation

Weighed about 1.0 g of Octane-1-sulphonicacidsodiumsaltin2000mlofwater, sonicate, and filtered through micron filter.

Mobile Phase - A

The mixed buffer solution and acetonitrile in the ratio of 90:10, adjusted pH of the mixture to 2.3 with Sulphuric acid, 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 and transferred about 5 mg of the Impurity-6 standard into a 100 ml volumetric flask, added 5 ml of acetonitrile, sonicated and dilute to volume with diluent.

System suitability Preparation

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

Standard Solution Preparation

About 75 mg of Milnacipran standard were weighed and then transferred to a 100 ml volumetric flask. The solution was then dissolved and diluted to a volume of 100 ml using diluent. Next, 5.0 ml of the solution was pipetted out into a 50 ml volumetric flask and further diluted to a volume of 50 ml using diluent.

Preparation of Test solution

Weighed accurately about 50 mg of Milnacipran sample and transferred to 50 ml of the volumetric flask, dissolved, diluted to 50 ml with diluent. The Optimized Chromatographic conditions is given Table

Table 1: Optimized Chromatographic Conditions

Column	C_{18}(100x4.6mm,2.7µ)
Elution Mode	Gradient
Flow Rate	1.0ml/min
Detection Wavelength	210nm
Injection Volume	10µL
Column Temperature	40°C
Analysis Run Time	75mins
Auto Sampler Temperature	10°C

Table 2: Gradient Run Time Program of the method

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
75.00	85	15

Validation of the developed method

The proposed method was validated as per ICH guidelines.

Results and Discussion

System suitability and specificity

In order to assess the appropriateness of the system, six duplicates of the standard solution preparation were injected, and variables such as theoretical plates, tailing factor, and percentage RSD were evaluated. By examining the spiked solution containing all impurities, it was shown that the blank and impurity peaks did not interfere with the active peak. The chromatograms of the blank and impurity-spiked solutions are shown in Figure, and the system appropriateness was determined to be well within the parameters. The findings are collated and provided in Table.

Table 3: Results of System Suitability Study

Parameter	Observation	Acceptance criteria
Tailing factor for Milnacipran Peak	1.23	NMT 2.0%
% Relative standard deviation for six replicate injections	1.34	NMT 5.0%
Resolution between Impurity-6 and Milnacipran HCl	6.82	NLT 2.0

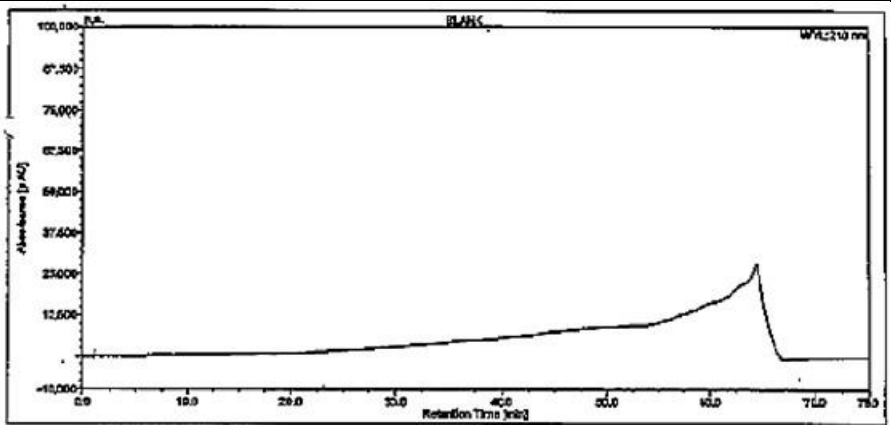

Figure 1: Chromatogram of Diluent (Blank)

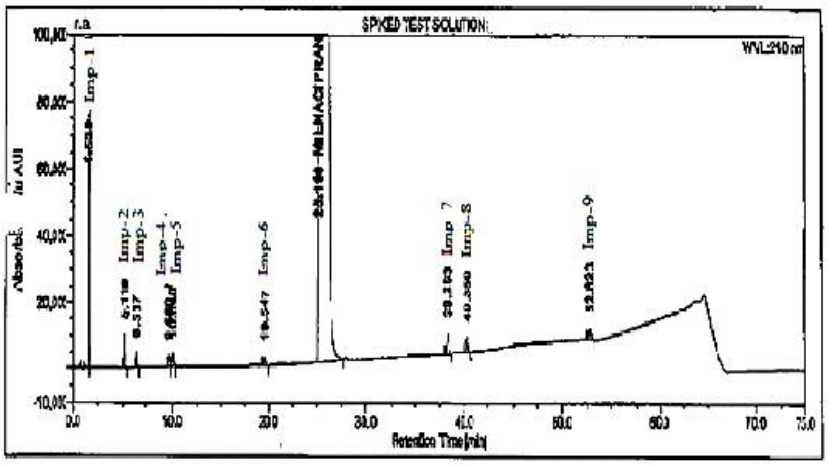

Figure 2: Chromatogram of Impurity spiked Solution

Limit of Detection and Quantification

By producing and analysing solutions of Milnacipran and its impurities at different concentrations, LOD and LOQ determination was carried out. After reading the answer, the corresponding chromatograms were recorded, and the mean areas were computed from the chromatograms. A graph illustrating the linearity between the concentrations and the mean peak area was created. Using the concentration vs mean areas of various levels graph, the limit of detection and quantification was computed. The results are listed in Table, and Figure displays the Limit of Quantification chromatogram.

Table 4: Results of Limit of Detection and Quantification Study

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

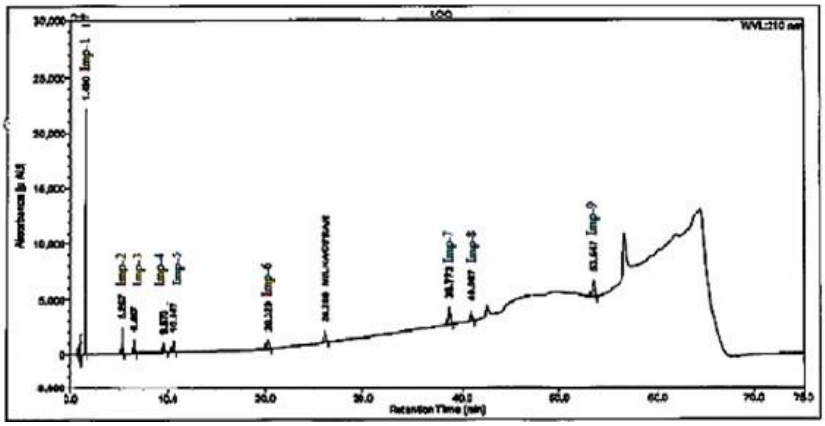

Figure 3: Chromatogram of Limit of Quantification

Linearity

In order to conduct a linearity research, various concentrations of contaminants and milnacipran (a spiking solution of impurities and milnacipran) were prepared spanning the range of the Limit of Quantification (LOQ) to 150% of the specification level. After injecting the ready solutions into liquid chromatography, the response was measured and related chromatograms were taken note of. A plot of concentration against mean peak area was created using the chromatograms to determine the mean peak area response. The regression approach was then used to get the intercept and correlation coefficient. The approach seems to be linear since the correlation coefficient result was well within the acceptable range of 0.997. Table displays the linearity data, while Figure displays the linearity plot of contaminants and millennacipran. In Figure, the linearity chromatogram is shown.

Table 5: Results of Linearity Study of Milnacipran and Impurities

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

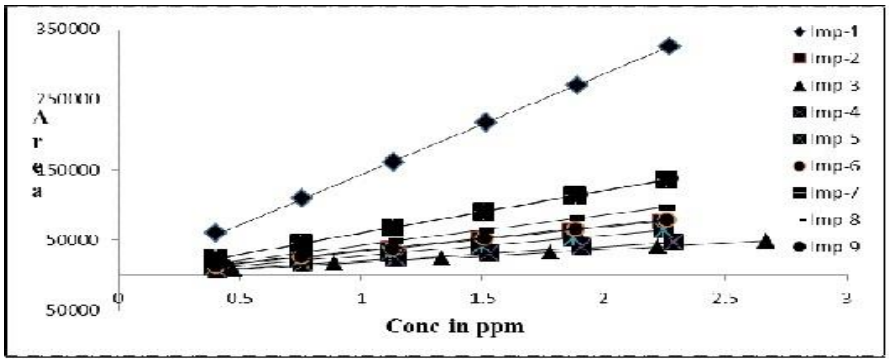

Figure 4: Linearity Plot of Milnacipran and impurities

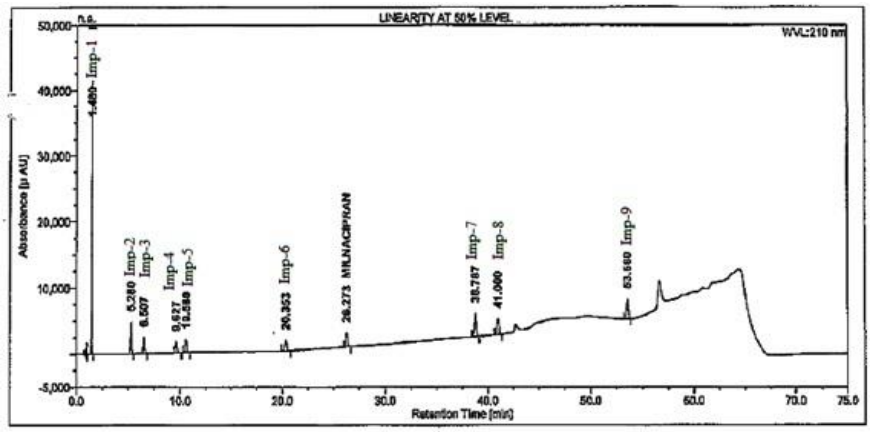

Figure 5: Chromatogram of Linearity of Milnacipran and impurities

Precision

Precision is "the degree of repeatability of an analytical method under normal conditions." The Precision of the method was measured by system precision, Method precision and Intermediate precision.

System Precision

In order to achieve system accuracy, a reference solution of milnacipran was prepared using an optimized technique, and six duplicates of the analysis were conducted in liquid chromatography. Chromatograms were recorded, and the area response was computed. After computation, the area response's percentage RSD was determined to be 0.23%, falling within the acceptable range of < 5.0%.

Method precision

The technique precision research included creating six distinct test preparations from a single batch and injecting them into liquid chromatography in accordance with the methodology. Each impurity's reaction was then measured, and the corresponding chromatograms were recorded. The accuracy of the proposed technique is shown by the percentage of organic impurities and the relative standard deviation of impurities of six independent analyses, which were computed and determined to be 0.32 % and within the acceptable range of < 5.0%.

Intermediate Precision (Ruggedness)

In order to demonstrate the method's repeatability, the investigation was conducted using an alternative column, instrument, and analyst on a separate day, resulting in six distinct test preparations from a single batch. The corresponding chromatograms were noted, the organic impurity % was examined, and the RSD of the impurity contents across six separate analyses was computed. The method's robustness is shown by the fact that it was determined to be within the acceptance threshold of ≤ 5.0%.

Accuracy Analysis (Recovery Analysis)

By creating a test solution in triplicate with known quantities of all contaminants at different concentrations (at LOQ, 100%, and 150% of concentration), the analytical method's accuracy was tested. Every solution that was introduced into the chromatograms and liquid chromatography was recorded. By comparing the regions in the Milnacipran sample before and after the addition of contaminants, the recovery was computed. The recovery fell between 91.02% to 103.43%, which is substantially within the acceptable range of 85% to 115%. This indicates that the approach is accurate for quantifying impurities. Table presents the recovery research data, whereas Figure shows the chromatogram.

Table 6: Results of Accuracy (Recovery Study) of impurities in Milnacipran

Level	Impurity-1	Impurity-2	Impurity-3	Impurity-4	Impurity-5	Impurity-6	Impurity-7	Impurity-8	Impurity-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

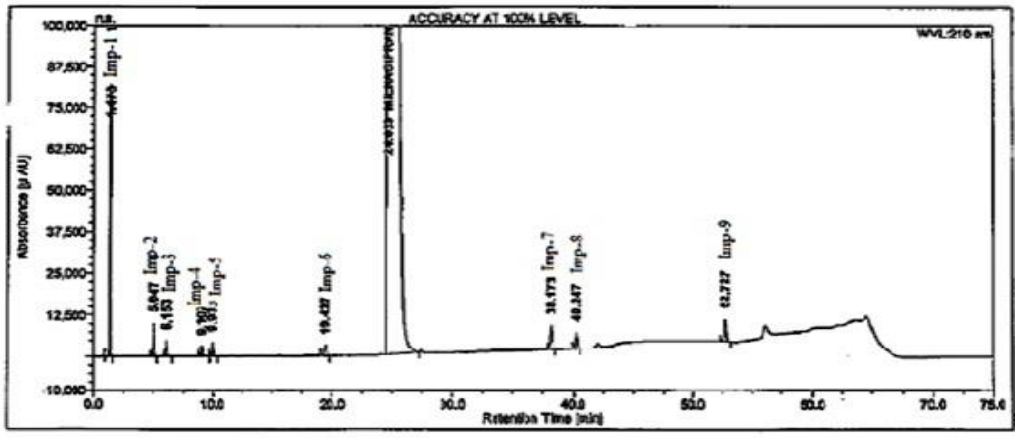

Figure 6: Chromatogram of Accuracy (Recovery) Study of Milnacipran

Stability of Analytical solution

Both the test solution and the impurity-spiked test sample were made, and they were kept at room temperature and 10°C to determine the stability of milnacipran in solution added to liquid chromatography both initially and for up to 48 hours at regular intervals. Chromatograms were made, responses were read, and the percentage difference between the test solutions' impurity peak area and the starting solution's was computed and compared. The percentage difference findings were determined to be within the tolerance of ± 2.0% and ranged from 0.2% to 1.8% over a period of 12 hours. The solution should be stored at 10°C for 12 hours to allow for the completion of the analysis, according to the findings.

Sturdiness

By proving the analytical method's dependability in the face of intentional chromatographic condition changes and by tracking its influence on system suitability parameters, its robustness was proven. In order to do this, optimised chromatographic settings were changed, including column temperature, pH of the buffer, mobile phase ratios, and test solution and standard analysis flow rates. Chromatograms were captured and the response was read. After analysing the observed system suitability parameter data, values were found to fall within the acceptable range.

Discussions on validation results

Because it is accurate in repeating findings and helps manage medication quality, the development of an analytical technique for impurity detection and quantification by chromatography has been mandated by regulatory agencies in many countries. According to ICH Guidelines, the suggested approach was validated. The findings of the specificity investigation were noted and showed that there was no interaction between Milnacipran and blank or its contaminants. The findings showed that the Limit of Quantification (LOQ) and Limit of Detection (LOD) ranged from 0.40 to 0.480 ppm and 0.003 to 0.017 ppm, respectively. The fact that the suggested technique produced the lowest LOD and LOQ values suggests how sensitive the devised method is.

There was a strong linear relationship (correlation coefficient: 0.9990) found between the concentration and matching impurity and Milnacipran peak regions. According to Table, the linearity ranged from 0.40 to 2.70 ppm. By examining the test solution laced with contaminants at different known concentrations, the accuracy (recovery) of all the impurities of the suggested approach was examined. By comparing the area of the Milnacipran sample before and after the addition of contaminants, the recovery was computed, and the findings ranged from 91.02% to 103.43%. The numbers were discovered to be within the acceptable range, demonstrating the current method's accuracy.

By creating and analysing six test preparations, the suggested method's precision was shown. The intermediate precision result was between 0.05% and 0.75 % RSD, and the technique precision was determined to be within a limit of 0.20 % to 0.60% RSD. The fact that the outcomes fell inside the acceptance criteria demonstrates the exact nature of the suggested approach.

The method's robustness was evaluated by intentional manipulation of different chromatographic settings. The system suitability criterion limitations, which demonstrate the current method's robustness, were not significantly impacted by the method's intentional modifications. Table presents an overview of the developed method's validation.

Table 7: Summary of validation of proposed analytical method

Sr. No.	Impurities	Linearity & Range(ppm)	Limit of Detection (ppm)	Limit of Quantification (ppm)	% Recovery Range (LOQ to 150% Level)
1	Impurity-1	0.404-2.271	0.003	0.008	103.39–101.38
2	Impurity-2	0.398-2.236	0.003	0.008	99.41–101.56
3	Impurity-3	0.474-2.669	0.006	0.018	99.30–101.91
4	Impurity-4	0.407-2.289	0.012	0.038	101.55–101.75
5	Impurity-5	0.400-2.248	0.001	0.017	93.58–103.43

6	Impurity-6	0.402-2.260	0.009	0.027	101.0–101.92
7	Impurity-7	0.401-2.258	0.003	0.01	91.02–99.03
8	Impurity-8	0.398-2.236	0.017	0.052	99.58–101.45
9	Impurity-9	0.404-2.270	0.014	0.042	101.09–103.05

Conclusion

The Summary proves that the proposed new method for quantification of the nine impurities in Milnacipran HCl is simple, sensitive, accurate, precise and robust in nature. The new developed method is a superior economical method which can be conveniently adapted in Quality control laboratories.

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