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Original Article

Novel RP-UPLC method development and validation for quantification of nefopam hydrochloride in tablet dosage form

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ABSTRACT

Objective: A simple, precise and accurate RP-UPLC method has been developed for the estimation of Nefopam hydrochloride in bulk and pharmaceutical dosage form.
Methods: The Chromatographic separation was achieved on C18 (4.6 mm * 100 mm; 3 microns) column using the mobile phase buffer: acetonitrile: methanol in the ratio of 50:41:9 at a flow rate of 0.6 ml/min. The detection wavelength was 225 nm and column temperature was set to 30°C.
Results: The retention time was found to be 2.6 minutes. System suitability parameters were studied by injecting standard six times and results were well under the acceptance criteria. Linearity was obeyed in the concentration of 22.63 µg/ml to 67.90 µg/ml and R^2 value was found to be 1.000. The recovery of Nefopam was found to be 99.72 %. In precision study % RSD was found to be 0.47 for repeatability and 0.60 % for intermediate precision. LOD and LOQ were 0.02 and 0.06 µg/ml respectively. By using the developed method, assay of marketed formulation was found to be 100.39%. The Beer's law was obeyed in the concentration of 22.63 ppm to 67.90 ppm.
Conclusion: The results of the study showed that the proposed RP-UPLC method was novel, simple, rapid, precise and accurate which is useful for the routine determination of Nefopam hydrochloride in bulk and pharmaceutical dosage form.

Introduction

Nefopam hydrochloride (Figure 1) ((±) – 3,4,5,6 – tetra hydro-5-methyl-1-phenyl-1H-2, 5-benzoxazocine hydrochloride) is a non-opioid analgesic and anti-inflammatory agent, chemically similar to the Orphenadrine [1]. It is widely used for the relief of moderate to severe pain. It is a white crystalline powder with an aqueous solubility of approximately 43.5 µg/ml at room temperature. It has log P of 3.16 and pka of 9 [2].

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Nefopam is metabolized by N-demethylation [3]. Nefopam inhibits the reuptake of serotonin, norepinephrine and dopamine. It also reduces glutamate signalling via modulating sodium and calcium channels [4]. Literature review revealed that Spectrophotometry [5-7], Thin Layer Chromatography [8], High Performance Liquid Chromatography [9-11], Liquid Chromatography-Mass Spectrometry [12], Gas Chromatography [13] methods have been developed for the quantification of Nefopam hydrochloride. Objective of the current study was to develop a novel RP – UPLC method for the quantitative determination of Nefopam hydrochloride in bulk and pharmaceutical dosage form as per ICH guidelines [14].

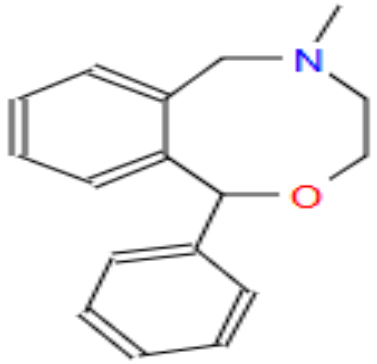

Figure 1: Structure of Nefopam.

UPLC

Ultra-Performance Liquid Chromatography (UPLC) is specially designed to withstand higher system pressures during chromatographic analysis so that it enables significant decreases in separation time and solvent consumption. The UPLC columns packed with 1.7 µm sized particles provides not only increased efficiency but also the ability to work at increased linear velocity without loss of efficiency but also the ability to work at increased linear velocity without loss of efficiency, providing both resolution and speed. Using advantages of UPLC, a number of applications in different fields including pharmacy, clinical analysis, pesticide analysis and tetracycline in human urine have been reported. The UPLC is based on the principle of use of stationary phase consisting of particles less than 2µm, while UPLC columns are typically filled with particles of 3 to 5 µm. The underlying principles of this evolution are governed by the Van Deemter equation, which is an empirical formula that describes the relationship between linear velocity (flow rate and plate height (HETP or column efficiency). The Van Deemter curve, governed by an equation with three components shows that the usable flow range for a good efficiency with a small diameter particle in much greater than for larger diameters [15-18].

Materials and Methods

Chemicals and Reagents

Nefopam hydrochloride standard was procured from Synthiya research laboratory, Pondicherry. Nefopam hydrochloride tablets (Nefosar-30 mg) were received as a gift sample from Cresent Laboratories Pvt. Ltd. Methanol HPLC grade, Acetonitrile HPLC grade, Triethylamine AR grade, and Orthophosphoric acid AR grade were obtained from Merck India Limited, Mumbai, and used for the preparation of mobile phase.

Instrumentation

Shimadzhu UV/VIS spectrophotometer with lab solution software was used for all the spectrophotometric measurements. The chromatographic estimation was performed on Agilent-1220 infinity UPLC system using Chemstation software. For pH adjustment of the solution, the Lab India pH meter was employed. Shimadzhu balance was employed for weighing the samples.

Diluent

Based up on the solubility of the drug, the diluent was selected. Buffer, acetonitrile and methanol were taken in the ratio of 50:41:9.

Preparation of standard stock solution

30 mg of Nefopam hydrochloride was accurately weighed and transferred to a 100 ml volumetric flask. Three-fourths of the diluent was added and sonicated for 10 minutes. The solution was made up to volume with diluent and labelled as standard stock solution.

Preparation of standard working solution (100% solution)

3.0 ml of Nefopam hydrochloride from the standard stock solution was pipetted into a 20.0ml volumetric flask and made up to volume with diluent.

Preparation of sample stock solution

Twenty tablets were accurately weighed, and the average weight of each tablet was calculated. Weight equivalent to 30 mg was transferred to a 100 ml volumetric flask. 50 ml of diluent was added, and the solution was sonicated for 25 minutes. The volume was then made up to 100 ml with diluent and filtered through 0.45µ syringe filters.

Preparation of sample working solution (100% solution)

3.0 ml of the filtered sample stock solution was transferred to a 20.0ml volumetric flask and made up to volume with diluent.

Preparation of buffer

2 ml of triethylamine was added to 500ml of HPLC water, and the pH was adjusted to 2.8 using OPA.

Method development

Based on the drug solubility and pKa value, the following conditions have been used to develop the method for the estimation of Nefopam hydrochloride.

Optimized chromatographic conditions

Column: C18; (4.6 mm * 100 mm); 3 microns
Mobile phase: Buffer: Acetonitrile: Methanol (50:41:9)
Buffer: 2 ml of triethylamine in 500ml of water, and pH adjusted to 2.8 using OPA.
Flow rate: 0.6 ml/min
Detector: 225 nm
Temperature: 30°C
Injection volume: 5 µl

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be presence of components that may be expected to be present. Typically, these might include excipients, impurities, degradants, etc. lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure [14].

Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results that are directly proportional to the concentration of analyte in the sample [14].

Accuracy

Twenty tablets were accurately weighed, and average weight of each tablet was calculated. Then, a weigh equivalent to 30 mg was transferred into a 100 ml volumetric flask. 50 ml of diluent was added, and the solution was sonicated for 20 minutes. The volume was made up with the diluent and filtered through 0.45µ syringe filters.

Acceptance criteria

The percentage recovery of each level should lie between 98.0 to 102%.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: Repeatability, intermediate precision, and reproducibility. The precision of an analytical procedure is usually expressed as the variance, standard deviations, or coefficient of variation of a series of measurements [14].

Robustness

Small deliberate changes in the method like flow rate, mobile phase ratio and temperature were made. However, there was no significant change in the results, and all values remained within the range of ICH guidelines. Robustness conditions like flow minus (0.5 ml/min), flow plus (0.7 ml/min), wavelength plus (226 nm) and wavelength minus (224 nm) were maintained and the samples were injected in duplicate. System suitability parameters were not significantly affected and %RSD remained within the acceptable limit.

LOD & LOQ

LOD & LOQ were calculated separately using the calibration curve method. The LOD and LOQ of the compound were determined using the developed UPLC method by injecting progressively lower concentrations of the standard solution.

Results and Discussion

Linearity

To demonstrate the linearity of the assay method, inject 6 standard solutions with concentrations ranging from 22.63 ppm to 67.90 ppm of Nefopam hydrochloride. Plot a graph of concentration versus peak area, and correlation co-efficient was found to be 1.000 (Table 1).

Table 1: Calibration data of nefopam hydrochloride.

Linearity level (%)	Concentration (ppm)	Area
50	22.63	406.490
70	31.69	570.096
100	45.27	811.991
125	56.58	1015.568
150	67.90	1212.887

Accuracy

Three concentrations of 80%, 100%, 150% were injected in triplicate, and manner and % recovery was calculated as 99.72% (Table 2).

Table 2: Accuracy data of Nefopam hydrochloride.

% level	Sample wt. (mg)	Area	Content (mg)	Content (%)
80%	174.35	657.582	29.849	99.50
	173.65	656.870	29.937	99.79
	175.55	655.822	29.566	98.55
100%	216.89	821.118	29.962	99.87
	216.38	818.532	29.938	99.79
	216.98	824.316	30.066	100.22
150%	259.36	983.982	30.025	100.08
	258.89	983.387	30.062	100.21
	261.01	983.886	29.833	99.44

Table 3: Repeatability data of Nefopam hydrochloride.

S. No.	Sample ID	Sample weight (mg)	Area	Content (mg)	% Assay
1	Sample 1	215.35	835.562	30.161	100.29
2	Sample 2	216.65	833.608	29.909	99.61
3	Sample 3	214.48	831.992	30.154	99.62
4	Sample 4	215.11	836.276	30.220	99.86
5	Sample 5	213.89	834.077	30.312	99.67
6	Sample 6	216.01	834.591	30.033	101.13
Mean				30.132	100.44
SD				0.142	0.472
%RSD				0.47	0.47

Precision

Repeatability

Six working sample solution of 45ppm were injected and the percentage amount was calculated. The % RSD was found to be 0.47 (Table 3).

Intermediate precision

Six working sample solutions of 45ppm are injected on the next day of the preparation of samples, and the % amount found was calculated. The % RSD was found to be 0.60 (Table 4).

Table 4: Intermediate precision data of Nefopam hydrochloride.

S. No.	Sample ID	Sample weight (mg)	Area	Content (mg)	% Assay
1	Sample 1	215.36	831.170	30.086	100.29
2	Sample 2	216.33	829.260	29.882	99.61
3	Sample 3	216.87	831.423	29.885	99.62
4	Sample 4	215.93	829.839	29.958	99.86
5	Sample 5	216.36	829.918	29.901	99.67
6	Sample 6	213.38	830.473	30.339	101.13
Mean				30.009	100.03
SD				0.179	0.597
%RSD				0.60	0.60

LOD

Detection limit of Nefopam hydrochloride in this method was found to be 0.02 µg/ml.

LOQ

Quantification limit of Nefopam hydrochloride in this method was found to be 0.06 µg/ml.

Robustness

Small deliberate changes in this method were made, such as flow minus, flow plus, wavelength plus and wavelength minus and the %RSD under these conditions was calculated (Table 5).

Table 5: Robustness data of nefopam hydrochloride.

Parameter	%RSD
Flow minus	0.33%
Flow plus	0.36%
Wavelength plus	0.30%
Wavelength minus	0.25%

Assay of marketed formulation

Standard solution (Figure 2) and sample solution were injected separately into the system and chromatograms were recorded, and drug present in the sample was calculated (Table 6).

Table 6: Assay of Nefopam hydrochloride.

S. No.	Standard Area	Sample Area	Label claim (mg)	Amount found (mg)	% Assay
1	805.191	808.198	100.39	30.01	100.39

System suitability

A standard solution of Nefopam hydrochloride working standard was prepared as per procedure and injected six times into the UPLC system. The system suitability parameters were evaluated from standard chromatograms obtained by calculating the % RSD of retention time, tailing factor, theoretical plates and peak areas from six replicate injections and were found to be within the range (Table 7).

Table 7: System suitability data of Nefopam hydrochloride.

S. No.	Drug	Retention time	Area	Theoretical plate	Tailing factor
1	Nefopam HCl	2.629	791.85	5994	1.242
2	Nefopam HCl	2.632	793.15	5885	1.239
3	Nefopam HCl	2.634	795.86	5819	1.267
4	Nefopam HCl	2.63	796.24	5901	1.251
5	Nefopam HCl	2.639	803.84	5789	1.239
6	Nefopam HCl	2.636	796.51	6018	1.267
Mean		2.63	796.24	5901	1.251
SD		0.004	4.164	91.49	0.013
%RSD		0.14	0.52	1.55	1.06

Conclusion

A novel RP- UPLC method was developed for the estimation of Nefopam hydrochloride in pharmaceutical dosage form. The method was optimized by evaluating various chromatographic conditions. Sample recovery was in good agreement with the respective label claim. The proposed method was validated as per ICH guidelines across all relevant parameters. This method can be effectively used for quality control testing of NSAID drug Nefopam hydrochloride in pharmaceutical dosage forms

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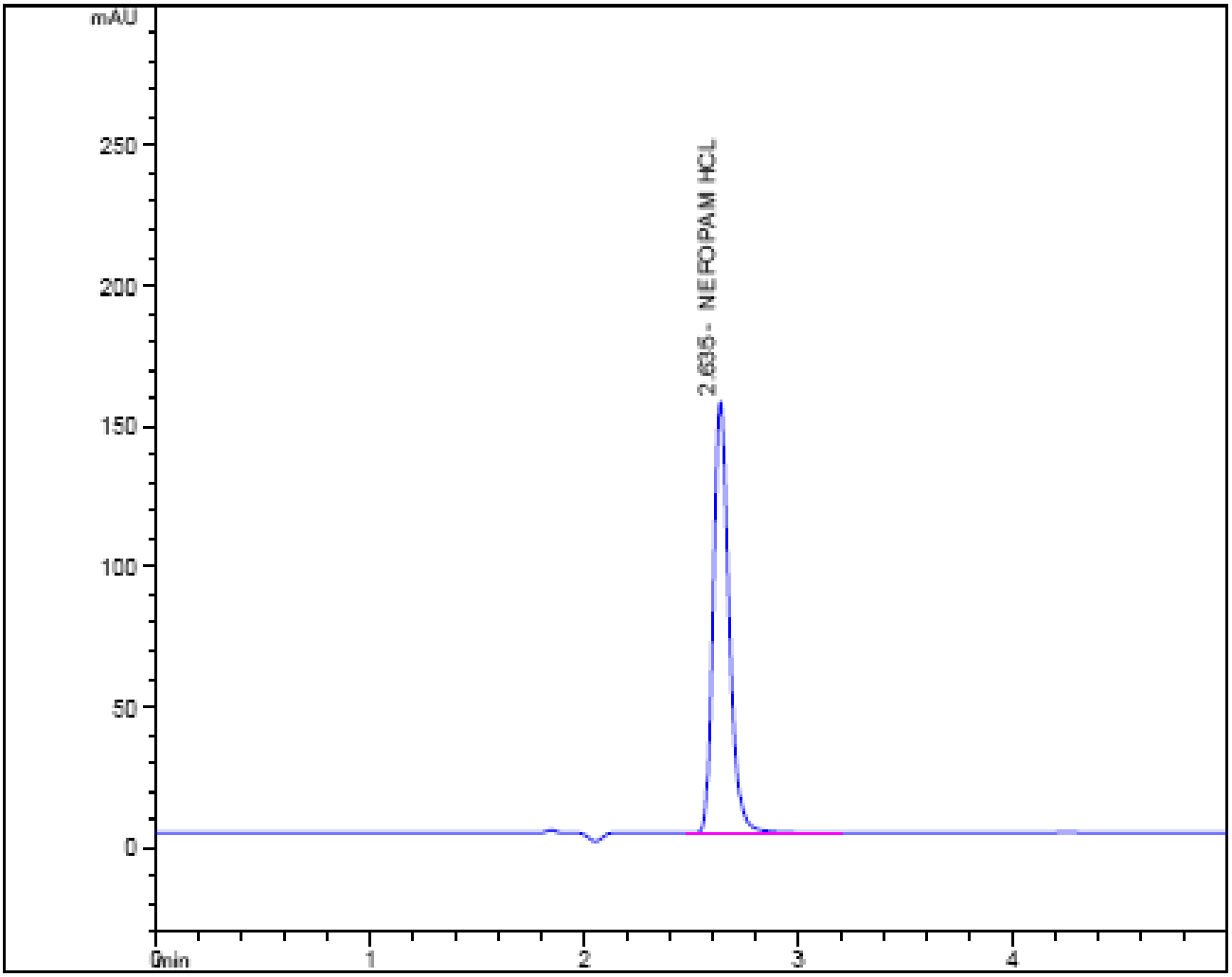

Figure 2: Chromatogram of Nefopam hydrochloride standard

Author Contributions

All authors contributed equally to this research. All authors read and approved the final manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

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Ethical Approvals

This study does not involve experiments on animals or human subjects.

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