International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 14, Issue 4, 2022

Original Article

A NOVEL METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTIFICATION OF NEFOPAM HYDROCHLORIDE IN PARENTERAL DOSAGE FORM BY RP-HPLC METHOD

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Received: 20 Apr 2022, Revised and Accepted: 12 Jun 2022

ABSTRACT

Objective: Using RP-HPLC, a simple, accurate, and exact method for estimating the Nefopam Hydrochloride parenteral dose form was devised.

Methods: The isocratic mode of the RP-HPLC method used an Inertsil C_8 column as the stationary phase and a mobile phase of potassium dihydrogen phosphate with pH 3.0: Acetonitrile (70:30) at a flow rate of 1 ml/min. With UV detection at 220 nm, a flow rate of 1 ml/min was established.

Results: The developed RP-HPLC technique revealed acceptable linearity (R2 = 0.9998) and good assay results in the concentration range of 0.004–0.08 mg/ml (103.3 percent). Further forced degradation investigations using 0.1N Hydrochloric acid (acid degradation), 0.1NNaOH (base degradation), and 3 percent H_2O_2 (Hydrogen peroxide) were carried out using RP-HPLC, and percent degradation values were determined. In peroxide degradation conditions, the medication was shown to be unstable.

Conclusion: In compliance with ICH requirements, the developed procedures were validated.

Keywords: Nefopam hydrochloride, RP-HPLC, Method development, Forced degradation studies

DOI: https://dx.doi.org/10.22159/ijcpr.2022v14i4.1982 Journal homepage: https://innovareacademics.in/journals/index.php/ijcpr

INTRODUCTION

Pharmaceutical analysis is particularly important in the quality assurance and control of bulk pharmaceuticals and pharmaceutical formulations. Due to the fast growth of the pharmaceutical business and medication production in many regions of the world, there is a greater demand for novel analytical techniques in the pharmaceutical industry. As a result, the most essential part of the analysis has become the development of analytical procedures. Analytical processes are designed and verified for active pharmaceutical ingredients (API), excipients, drug products, degradation products, related substances, residual solvents, and other chemicals. In quality control laboratories, these analytical methods are used to assure pharmaceuticals' identity, purity, safety, efficacy, and performance [1].

Nefopam Hydrochloridehas the empirical formula $C_{17}H_{19}NO$. HCl and with the IUPAC name 3,4,5,6-Tetrahydro-5-methyl-1-1-phenyl 1H-2, 5-hydrochloride, 5-Methyl-1-phenyl-1, 3, 4, 6-tetrahydro-5H-benz[f]-2,5-oxazocine hydrochloride [2], is a non-opioid analgesic that inhibits the reuptake of serotonin, dopamine, and noradrenaline. Nefopam is a painkiller. It treats moderate pain, for example, after an operation or a serious injury, dental pain, joint pain, muscle pain, or pain from cancer [3]. This drug is therapeutically used for the relief of moderate to severe pain. The structure of Nefopam hydrochloride is shown in fig. 1[4].

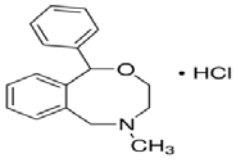

Fig. 1: Structure of nefopam hydrochloride

RP-HPLC is a precise and sensitive method for analyzing the quantitative effects of a variety of medicines. A few approaches for innovative method development and validation for the measurement of nefopam hydrochloride in parenteral dose form have been documented in the literature. The goal of this study was to create and validate Nefopam Hydrochloride using RP-HPLC and conduct forced degradation studies [5].

MATERIALS AND METHODS

The tools used to analyze Nefopam Hydrochloride are listed in table 1.

Table 1: List of instruments/apparatus used

S. No.	Name	Date handling system	Make	Model
1	Analytical Balance	-	Shimadzu	AY220
2	Digital pH-Meter	-	Lobotronics	LT-11
3	Sonicator	-	Enerteck	-
4	UV-Visible Spectrophotometer	UV probe	Shimadzu	UV-1800
5	High-Performance Liquid Chromatography	LC-Solution	Shimadzu	LC-2010
6	Millipore vacuum filtration setup (0.45 µm)	-	-	-
7	Calibrated electronic balance	-	Sartorius	-
8	Volumetric flask (10, 20,25,50 ml)	-	Class borosil	-
9	Pipettes (1 micro pipette), 2,5,10 ml	-	Class borosil	-
10	Beakers (50, 100 ml)	-	Class borosil	-

Active pharmaceutical ingredient used

The Nefopam Hydrochloride was obtained from Aurobindo Pharma Ltd. The marketed formulation Accupan®, Aurobindo Pharma Ltd. Hyderabad, India was used as a parenteral dosage form.

Chemicals used

The following table 2 shows the list of chemicals used for the analysis of Nefopam.

Methodology

Selection of detection wavelength

20 mg of Nefopam Hydrochloride was dissolved in water. The spectrum was acquired by scanning the solution from 200 to 400 nm. The overlay spectrum was used for the selection of wavelength for Nefopam Hydrochloride. The detecting wavelength was chosen as the isosbestic point.

Table 2: List of chemicals used

S. No.	Name	Manufacturer
1.	Distilled water	In-House Production (Sri Padmavathi School Pharmacy)
2.	Hydrochloric Acid	Merck Pvt. Ltd (AR Grade)
3.	Sodium Hydroxide	Merck Pvt. Ltd (AR Grade)
4.	HPLC grade Acetonitrile	Merck Pvt. Ltd (AR Grade)
5.	HPLC grade water	Merck Pvt. Ltd (AR Grade)
6.	Ammonium acetate	Merck Pvt. Ltd (AR Grade)
7.	Potassium Dihydrogen phosphate	Merck Pvt. Ltd (AR Grade)
8.	Orthophosphoric acid	Merck Pvt. Ltd (AR Grade)
9.	Hydrogen peroxide	Spectrum chemicals
10.	Methanol	Merck Pvt. Ltd (AR Grade)
11.	Ethanol	Spectrum chemicals
12.	Acetone	Spectrum chemicals

Selection of column

As the drug is polar, initially, nonpolar column Symmetry®C_{18} (250*4.6 mm)–5 µm is used. It offers good peak symmetry.

Selection of mobile phase

As the drug is freely soluble in water, it has been selected as one of the solvents. Ammonium acetate and potassium dihydrogen phosphate are used as buffers as their pH correlates to the drug pka value. Acetonitrile is employed to generate a strong peak since the medication molecule is polar and basic.

Selection of pH of mobile phase

Nefopam hydrochloride is basic, and a tertiary amine group is present in it. As it is a bulker molecule, acidic range pH is selected from 3-5. When the pH of the drug is increased, its ionization will be increased.

Selection of diluent

Water was used as the diluent because of the drug's solubility.

Selection of flow rate

The flow rate should not be more than 2 ml/min. The flow rate that results in the shortest retention period, the lowest back pressures and the best separation will be chosen.

Preparation of solutions

- Preparation of Nefopam Hydrochloride Standard stock solution-I (0.4µg/ml): 20 mg of Nefopam API was weighed and transferred into a 50 ml volumetric flask, dissolved in a freshly prepared diluent, and made up to the volume.
- Preparation of Nefopam Hydrochloride Standard stock solution-II (0.04µg/ml): 5 ml was pipetted out from stock-I into a 50 ml volumetric flask and made up to the mark with the diluent.
- Preparation of Nefopam Hydrochloride Sample stock solution-I (0.4µg/ml): 2 ml of Nefopam sample was transferred into a 20 ml volumetric flask, dissolved in a freshly prepared diluent, and made up to the volume.
- Preparation of Nefopam Hydrochloride Sample stock solution-II (0.04µg/ml): 2 ml was pipetted out from stock-I into a 50 ml volumetric flask and made up to the mark with the diluent.

Optimization of Chromatographic conditions

Method development for the analysis of Nefopam Hydrochloride was done by changing mobile phase ratios, buffers, flow rate, columns, and run time. The chromatogram is shown in fig. 2.

Table 3: Optimized chromatographic conditions

S. No.	Parameters	Condition
1	Column	Intersil ® C_8-3(250*4.6 mm)-5 µm
2	Pump	LC-10
3	Pump mode	Isocratic
4	Mobile phase	Potassium dihydrogen phosphate: Acetonitrile (70:30)
5	Detection wavelength	220 nm
6	Flowrate	1.0 ml/min
7	Standard and sample concentration	0.04 mg/ml
8	Column oven temperature	30 ° C
9	Run time	20 min
10	Injection volume	10 µl

Table 4: Results of nefopam hydrochloride optimised method

Name	RT	Area	TP	TF
NEFOPAM	6.853	2058146	7120	1.7

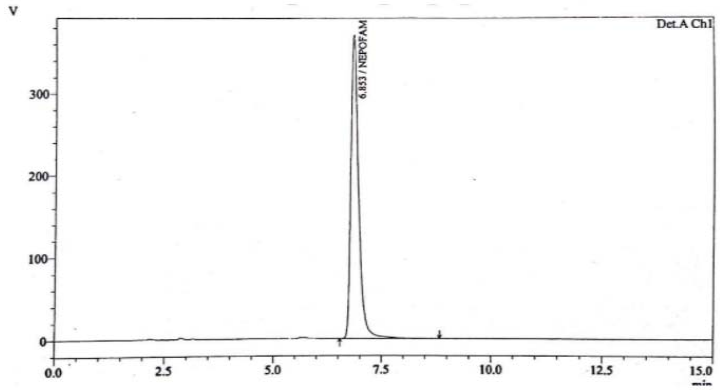

Fig. 2: Chromatograms of the optimized method

Inference

In this trial, theoretical plates and tailing factors are within the limit; hence this trial was optimized.

Method validation by RP-HPLC

Validation was performed as per the ICH Q2B (R2) guidelines [6, 7]. The method was validated for the parameters like system suitability, specificity, linearity, precision (system precision and repeatability), accuracy, the limit of detection and quantification, robustness, and assay. The stability studies like acid degradation, base degradation, and degradation with hydrogen peroxide were carried out as per ICH guidelines [8].

System suitability parameters [9]

It is performed to verify that the analytical system is working properly and can give accurate and precise results. 5 injections of Nefopam Hydrochloride Standard solution (0.04 mg/ml) were injected into HPLC and system suitability parameters like USP theoretical plate count, and tailing factor, were assessed, which was mentioned in fig. 3.

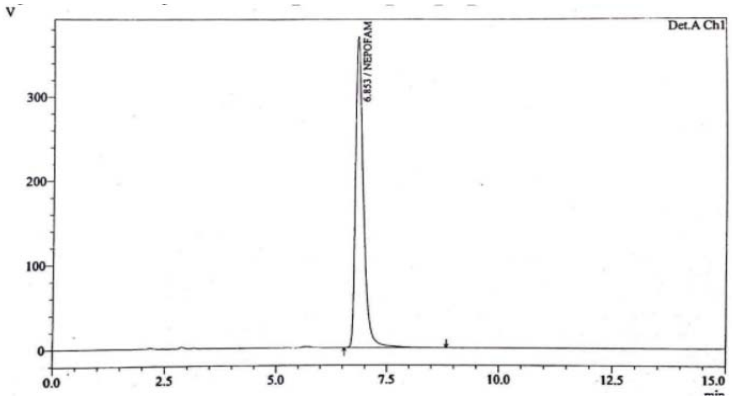

Fig. 3: Chromatogram of system suitability

Table 5: System suitability parameters for nefopam hydrochloride

Inj. No.	RT	Peak area	Theoretical plates	USP tailing factor
1	6.853	1906390	8945	1.2
2	6.853	1906175	8990	1.2
3	6.853	1905960	9053	1.2
4	6.825	1904806	9074	1.2
5	6.824	1903697	9096	1.2
Mean		1905405		
SD		1134.3735		
%RSD		0.06		

Acceptance criteria

- Theoretical Plates–NLT 2000
- USP Tailing Factor–NMT 2.0
- % RSD–NMT 2.0

Discussion

Because the system suitability parameters were within the limitations, the parameters for the optimized method could be used to validate the method.

Specificity

The specificity of the method is performed by separately injecting the blank, placebo, and sample solutions. In the improved approach, the interference is checked. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific.

Discussion

From the above chromatogram, it can be concluded that there is no interference between the blank and placebo in the method.

Linearity

Weigh accurately and transfer about 20.0 mg of Nefopam Hydrochloride working standard into 50 ml volumetric flask, add 20 ml of diluent, sonicate with intermittent shaking to dissolve the contents, and dilute to 50 ml with diluent and mix well. Further, dilute 5 ml of this solution to a 50 ml volumetric flask and dilute to volume with a diluent.

Standard solutions of 50%, 100%, and 150% concentrations were prepared by taking 0.5, 1.0, and 1.5 ml each from two standard stock solutions to make up to 10 ml.

Linearity for the concentration range 0.004-0.08 mg/ml was established by plotting concentrations mentioned in fig. 7.

Discussion

Six linear concentrations of Nefopam (50-300 µg/ml) were injected in a duplicate manner. Average areas were mentioned above, and linearity equations obtained for Nefopam was y = 7189x+28445; the correlation coefficient obtained was 0.999.

Precision

Preparation of solutions

Preparation of Nefopam Hydrochloride Standard stock solution-I (0.4 mg/ml): 20 mg of Nefopam API was weighed and transferred into a 50 ml volumetric flask and dissolved in freshly prepared diluent and made up to the volume.

Preparation of Nefopam Hydrochloride Standard stock solution-II (0.04 mg/ml): 5 ml was pipetted out from stock-I solution into 50 ml volumetric flask and made up to the mark with the diluent.

Preparation of Nefopam Hydrochloride Sample stock solution-I (0.4 mg/ml): 2 ml of Nefopam sample was transferred into a 20 ml volumetric flask and dissolved in freshly prepared diluent and made up to the volume.

Preparation of Nefopam Hydrochloride Sample stock solution-II (0.04 mg/ml): 2 ml was pipetted out from the stock-I solution into a 50 ml volumetric flask and made up to the mark with the diluent.

Method precision: 6 individual sample preparations are injected into the system.

Table 6: Linearity profile by RP-HPLC

Concentration (µg/ml)	Peak area of nefopam
0	0
50	488555
100	995266
150	1493280
200	2020341
250	2492454
300	3036166

Table 7: Summary of regression equation by RP-HPLC

Line equation	y = 7189x+28445
Correlation coefficient (R^2)	0.999
y-intercept (C)	3030.8
Slope (m)	7189

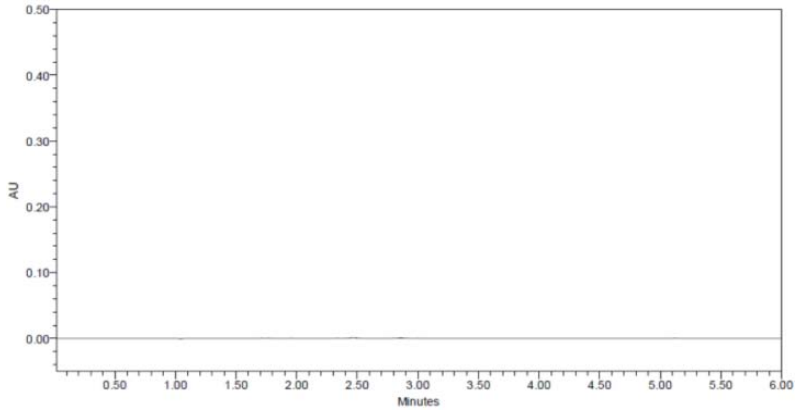

Fig. 4: Chromatogram of blank

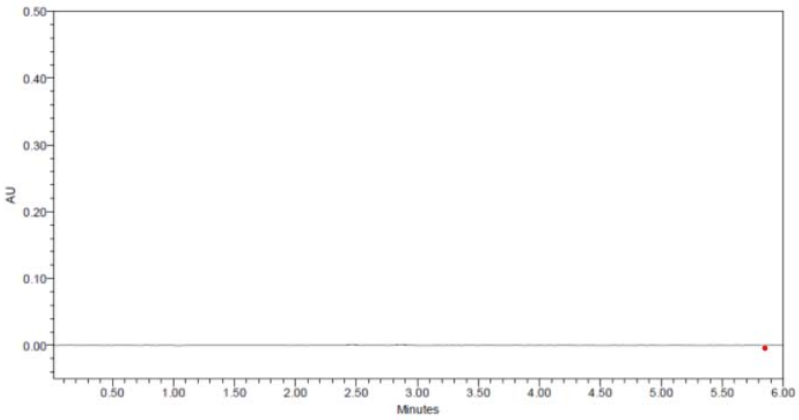

Fig. 5: Chromatogram of placebo

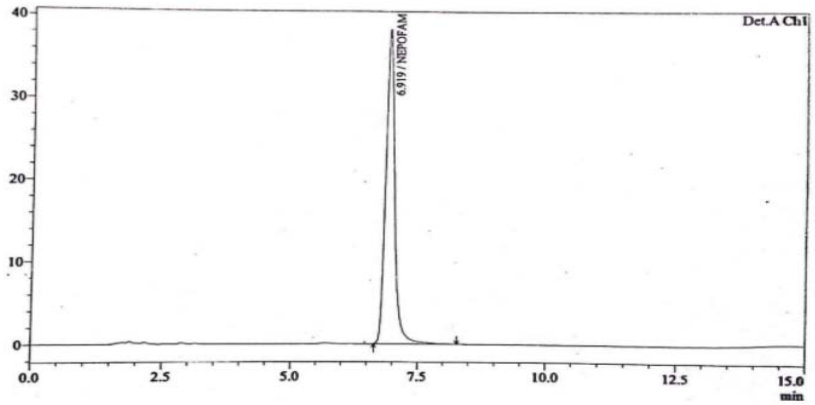

Fig. 6: Overlay chromatograms of blank, placebo, and nefopam hydrochloride

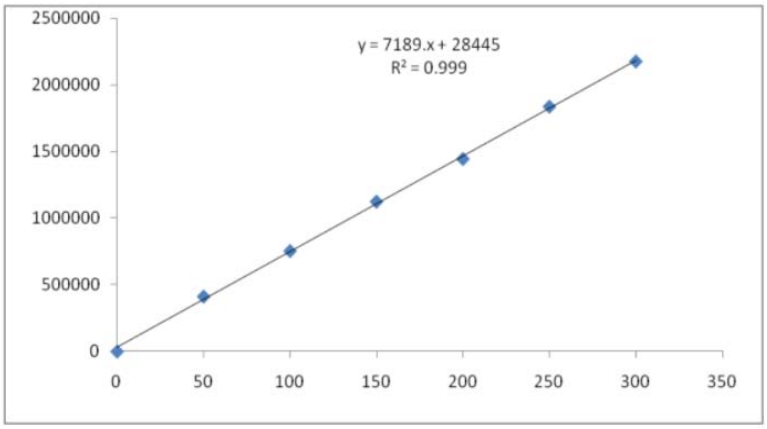

Fig. 7: Calibration curve of Nefopam HCl, On X-axis and the corresponding peak area on Y-axis. Statistical parameters like correlation Coefficient (R^2), line equation including slope (m), and y-intercept (C) were determined

Table 8: Results for precision by RP-HPLC

Sample name	Volume taken (ml)	Dilution	Peak area	Mg/ml	% assay (w/w)
NFM-PREP-1	2	50	1972416	10.3	103.2
NFM-PREP-2	2	50	1973948	10.3	103.2
NFM-PREP-3	2	50	1975734	10.3	103.3
NFM-PREP-4	2	50	1974810	10.3	103.3
NFM-PREP-5	2	50	1978904	10.3	103.5
NFM-PREP-6	2	50	1980098	10.4	103.6
Average-103.3, SD-0.154, % RSD-0.1					

Discussion

From a single volumetric flask of working standard solution, six injections were given, and the obtained areas were mentioned above. Average area, standard deviation, and % RSD were calculated. % RSD obtained as 0.1 %respectively for Nefopam. As the limit of Precision was less than "2" the system precision was passed in this method.

Accuracy

Standard preparation (0.04 mg/ml)

Transfer about 20.0 mg of Nefopam Hydrochloride working standard to a 50 ml volumetric flask, add 20 ml of diluent, sonicate with intermittent shaking to dissolve the contents, then dilute to 50 ml with diluent and mix it well. Combine 5 ml of this solution with 50 ml of water to make a 50 ml solution. Fill a volumetric flask halfway with diluent and dilute to volume.

Preparation of 50% Spiked solution: In a 10 ml volumetric flask, 0.5 ml of sample stock solution was pipetted out, and 1.0 ml of each standard stock solution was pipetted out and made up to the mark with diluent.

Preparation of 100% Spiked solution: 1.0 ml of sample stock solution was pipetted into a 10 ml volumetric flask, along with 1.0 ml of each standard stock solution, and diluent was added to make up to the mark.

Preparation of 150% Spiked solution: 1.5 ml of sample stock solution was pipetted into a 10 ml volumetric flask, followed by 1.0 ml of standard stock solution pipetted out and diluent prepared up to the mark.

Placebo solution of 0.2 ml was spiked with 50,100.150% level solutions of standard stock solution and analyzed. 10 µl of each of the above solutions were injected into the chromatographic system and peak areas were noted. Calculated the individual recovery and mean recovery values.

Acceptance criteria

Each level's percent recovery should be between 98.0 and 102.

Table 9: Accuracy results for nefopam hydrochloride

Recovery levels	Standard volume taken	Dilution	Area	Amount added (mg/ml)	Amount found (mg/ml)	% Recovery
50%	2.5 ml	50	1036562	0.021	0.021	103.6
100%	5 ml	50	2024827	0.042	0.042	101.2
150%	7.5 ml	50	3042072	0.063	0.063	102.1
Average-102.1, SD-1.3495, % RSD-1.3						

Discussion

Three levels of Accuracy samples were prepared by the standard addition method. Triplicate injections were given for each level of accuracy and mean % Recovery was obtained as 101 % for Nefopam.

Limit of detection and limit of quantification

The LOD and LOQ of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. "LOD and LOQ are related to both the signal and the noise of the system and is usually defined as a peak whose signal-to-noise (S/N) ratio are at least 3:1 for LOD and 10:1 for LOQ" [10]. The results were shown in table 10.

Robustness

Typical variations includea change in flow rate (±0.1 ml of optimized flow rate), change in pH of the buffer (±10%), and column oven temperature (25 °C and 35 °C) was assessed.

Discussion

Robustness conditions like Flow minus (0.9 ml/min), Flow plus (1.1 ml/min), temperature minus (25 °C), and temperature plus (35 °C) were maintained, and mobile phase pH (2.9 and 3.1) samples were injected in a duplicate manner. System suitability parameters were not much affected, and all the parameters were passed. % RSD was within the limit.

Solution stability

Sample and Standard solutions are prepared at the method concentration (0.04 mg/ml) and are injected for 2,4,8,12,15,20,24 h time intervals their peak areas were noted and % variation is calculated.

Table 10: Results for lod and loq of aclidinium bromide and formoterol fumarate

Drug	LOD (µg/ml)	LOQ(µg/ml)
Nefopam hydrochloride	0.0014	0.0043

Table 11: Summary of robustness data

Parameter	Condition	System suitability parameters		% RSD
		Theoretical plates	USP tailing factor	
Change in flow rate (±0.1 ml/min)	0.9 ml/min	9134	1.2	0.08
	1.1 ml/min	8213	1.2	0.04
Change in temperature (25 °C and	25 °C	8844	1.2	0.04
35 °C)	35 °C	8959	1.2	0.06
Change in Buffer pH (2.9,3.1)	2.9	8444	1.2	0.05
	3.1	8958	1.2	0.05

Table 12: Sample solution stability

Solution stability time	Nefopam hydrochloride	
	Peak area	% Variation
Initial	1982592	NA
2 h	1986145	0.17
4 h	1985929	0.18
8 h	1984987	0.23
12 h	1983594	0.30
15 h	1983315	0.32
20 h	1982592	0.35
24 h (Benchtop)	1982592	0.78

Table 13: Standard solution stability

Solution stability time	Nefopam hydrochloride	
	Peak area	% Variation
Initial	1934233	NA
2 h	1922769	0.59
4 h	1920096	0.73
8 h	1919829	0.74
12 h	1919829	0.90
15 h	1914941	1.00
20 h	1914787	1.01
24 h (Benchtop)	1904788	1.53

Assay of formulation by RP-HPLC

Calculated the content of Nefopam Hydrochloride by using the following formula.

Content in mg/ml

$$\frac{PeakArea}{Mean} \times \frac{StandardWeightTaken}{StandardDilution} \times \frac{StandardVolumeTaken}{StandardDilution} \times \frac{SampleWeightTaken}{Dilution} \times \frac{SampleVolumeTaken}{Dilution} \times Potency \times 100$$

$$= \frac{1972416}{1905405} \times \frac{20}{50} \times \frac{5}{50} \times \frac{2}{20} \times \frac{2}{50} \times 99.9 \times 100$$

= 10.3 mg/ml

$$\%Assay = \frac{Content\left(\frac{mg}{ml}\right)}{Labelclaim} \times 100$$

= 103.2%

Acceptance criteria: 90-100 %. Assay results were satisfactory and found to be within the limits.

Forced degradation studies

Preparation of solution

Preparation of 1N Hydrochloric acid: 0.85 ml of HCL was taken in a 10 ml volumetric flask and made up to the mark with carbon dioxide-free water.

Preparation of 1N Sodium Hydroxide: 0.40g of NaOH was taken in a 10 ml volumetric flask and made up to the mark with carbon dioxide-free water.

Preparation of 3% Hydrogen peroxide: 10 ml was taken in a 100 ml volumetric flask from 30% hydrogen peroxide and made up to the mark with water.

Acid degradation

1 ml of sample solution was pipetted into a 10 ml volumetric flask and added 1 ml of 1N HCl. Then kept in a hot air oven at 60 °C for 3 h. After 3hours the flask is taken out and added 1 ml of 1N NaOH and made up to the mark with the diluent. From the above solution, 2 ml was pipetted out into a 50 ml volumetric flask and made up to the mark with diluent, and then injected into HPLC.

Base degradation

1 ml of sample solution was pipetted into a 10 ml volumetric flask and added 1 ml of 1N NaOH. Then kept in a hot air oven at 60 °C for 3 h. After 3 h the flask is taken out and added 1 ml of 1N HCl and made up to the mark with the diluent. From the above solution, 2 ml was pipetted out into a 50 ml volumetric flask and made up to the mark with diluent, and then injected into HPLC.

Degradation with hydrogen peroxide

1 ml of sample solution was pipetted into a 10 ml volumetric flask and added 1 ml of 3% Hydrogen peroxide, made up to the mark with diluent, and then injected into HPLC.

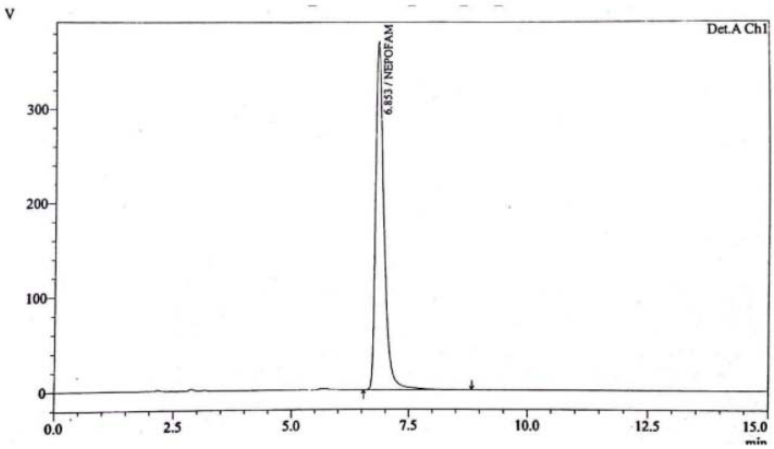

Fig. 8: Acid degradation (0.1 N HCl)

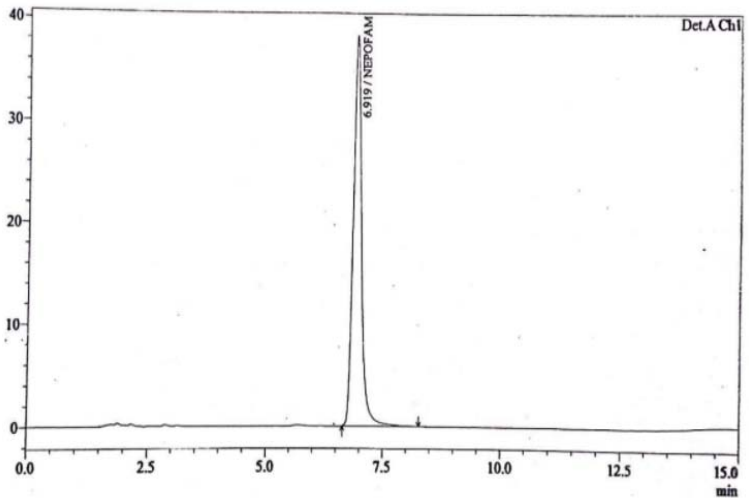

Fig. 9: Base degradation (0.1 N HCl)

Table 14: Calculation of forced degradation studies

Condition	Area	% Degradation
Basic condition	1902678	0.19
Acidic condition	1896187	0.53
Hydrogen peroxide	1868844	1.96

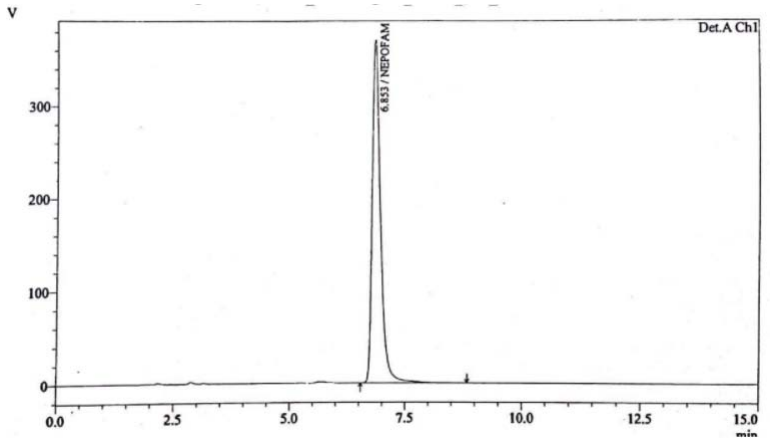

Fig. 10: Peroxide degradation (0.1 N HCl), The above fig. are the chromatograms of acid, base, and peroxide degradation studies.

DISCUSSION

Forced degradation studies reveal the % degradation values of Nefopam Hydrochloride, and the drug was found to be unstable at peroxide conditions.

CONCLUSION

Analytical method RP-HPLC was developed and validated to analyze Nefopam Hydrochloride in the parenteral dosage form [11].

- The developed RP-HPLC method for quantification of Nefopam Hydrochloride was found to be accurate, precise, and robust.[12]

- The forced degradation studies in RP-HPLC were performed and % degradation values are determined. The drug was found to be unstable in peroxide degradation conditions.

RP-HPLC method in isocratic mode involved the utilization of Intersil C_8 column as stationary phase and mobile phase constituting potassium dihydrogen phosphate with pH 3.0: Acetonitrile (70:30) at a flow rate of 1 ml/min with UV detection at 220 nm was developed. The developed RP-HPLC method demonstrated good linearity in the concentration range of 0.004-0.08 mg/ml (R_2= 0.9998) and good assay results (103.3%). Further forced degradation studies were carried out by RP-HPLC using 0.1N HCL (acid degradation), 0.1N NaOH (Base degradation), and 3%H_2O_2 (Hydrogen peroxide), and % degradation values are determined. The drug was found to be unstable in peroxide degradation conditions.

All the methods were validated as per the ICH guidelines and can be used for regular analysis of Nefopam Hydrochloride in Quality Control Laboratories.

ACKNOWLEDGMENT

The author wishes to thank AUROBINDO PHARMA Pvt Ltd., Hyderabad, for providing the facility to complete the work.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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