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Analysis Of Nefopam Hydrochloride From Bulk And Dosage Form Using Validated Rp Hplc Method

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ABSTRACT

Chemistry-basedanalytical methods play a vital role in the process of identification, separation, and quantification of chemical components present in natural or synthetic materials. The main purpose of analytical method development and validation is to prove that the proposed analytical method is accurate, specific, precise, and robust and can be applied in the pharmaceutical industry for the analysis of a drug moiety. Analytical evaluation provides important information about the potency of a drug, its bioavailability, stability, and itsin-vivo fate.

Method: *Here, a novel RP-HPLC method has been developed and validated for the evaluation of nefopam hydrochloride in bulk and dosage form. The method was validated and analyzed statistically forsystem suitability, specificity, and sensitivity, linearity and range, accuracy precision, filter study, solution stability, and robustness as per ICH guidelines.*

Result: *The retention time of nefopam hydrochloride was around 5.313 min. The percentage RSD of each parameter was found within the limit. The recovery of nefopam hydrochloride was found to be 100.4%. The method was linear over the range of 16-120 µg/ml with a regression coefficient of 0.999. All the other verification parameters were within the range according to ICH guidelines.*

*C**onclusion:**The developed method can be successfully employed for accurate, precise, and reliable estimation of nefopam hydrochloride from bulk and formulation.*

Keywords: *Nefopamhydrochloride, RP-HPLC, accuracy, robustness, theoretical plates.*

INTRODUCTION

Chromatography is a powerful separation method that finds application in all branches of science for analytical purposes. High-Performance Liquid Chromatography or High-Pressure Liquid Chromatography (HPLC) is a chromatographic technique that can separate a mixture of compounds and is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of a mixture of analytes by partitioning between the mobile phase (a flowing liquid) and a stationary phase (sorbents packed inside a column).

Nefopam hydrochloride is a potent, rapidly acting non-narcotic analgesic that is used to treat moderate pain, for example after an operation or a serious injury, dental pain, joint pain or muscle pain, or pain from cancer [1-3] It is a white crystalline powder with an aqueous solubility of approximately 43.5 ug/mL at room temperature. It has a log P of 3.16, pKa of 9 [4].Very few analytical methods for the evaluation of nefopam

hydrochloride in bulk and dosage form have been reported in the literature [5,6]. This study endeavors to develop and validate a novel RP HPLC method for the evaluation of nefopam hydrochloride (Figure 1). Its IUPAC name is 5-methyl-1-phenyl-1,3,4,6-tetrahydro-2,5-benzoxazocine; hydrochloride, molecular formula is $C_{17}H_{20}ClNO$ and molecular weight- is 289.8 g/mol (4).

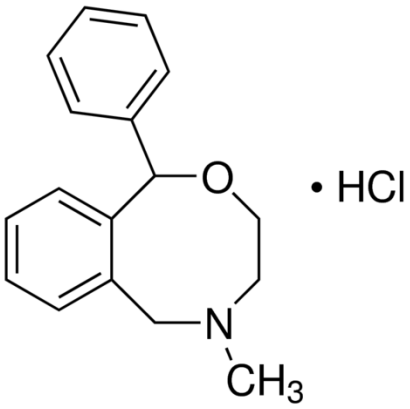


Fig.1: Nefopam hydrochloride

MATERIAL AND METHODS

Chemicals and Reagents: An analytically pure nefopam hydrochloride standard was procured from the Central Drugs Testing Laboratory, Mumbai.Nefopam tablets 30 mg were received as a gift sample from Torrent Laboratories Pvt. Ltd.Acetonitrile HPLC grade, methanol HPLC grade, anhydrous-1-hexanesulphonic acid Na salt HPLC grade, triethylamine AR grade, and orthophosphoric acid AR gradewere obtained from MerckIndia Limited, Mumbai,and used for the preparation of mobile phase.

Instrumentation

Perkin Elmer UV/VIS Spectrometer Lambda 25 connected to a computer loaded with software Perkin Elmer UV Win Lab was used for all the spectrophotometric measurements. The chromatographic estimation was performed on Perkin Elmer Flexar HPLC using software TC Nav/ver 6.3.2 with LC instrument control. An Inert Waters C18 (250 mm × 4.6 mm×µ5m) column was used as a stationary phase.Meltronics sonicator was usedto enhance the solubility of the drugs. For pHadjustment of the solution, the Elico pH meter wasemployed. Sartorius balance was employed for weighingthe samples.

Preparation of Buffer pH 2.7 for Mobile Phase

Dissolve 1.88 gm of anhydrous 1-hexanesulfonic acid sodium salt in 1000 ml of water. Add 2.0 ml of triethylamine. Adjust to pH 2.7 ± 0.05 with concentrated phosphoric acid. Filter through 0.45µm nylon membrane filter.

Preparation of Mobile Phase

Mix buffer pH 2.7, acetonitrile, and methanol in the ratio 55:30:15.Sonicate to degas.

Preparation of Diluent

Diluent I: Water: acetonitrile 50:50 (V/V)
Diluent II: 0.1 N hydrochloric acid.

Preparation of Blank Solution

Use Diluent II as a blank solution.

Preparation of Standard Solution (60 ppm)

Weigh and transfer accurately 30 mg of nefopam hydrochloride working standard into 25 ml. volumetric flask. Add 15 ml. of diluent I, sonicate to dissolve with intermediate shaking, and makeup volume with diluent I and mix.Further, dilute 5 ml of above standard solution in 100 ml with diluent II and mix.

Preparation of Sample Solution (60 ppm)

Weigh and crush ten intact tablets (equivalent to 300 mg of nefopam hydrochloride) and transfer them to a previously dried 250 ml volumetric flask. Add 150 ml of diluent I and sonicate for 15 minutes with intermediate shaking. Makeup volume with diluent I and mix. Filter through 0.45 µm nylon filter; discard

first 5 ml. of filtrate.Further, dilute 5 ml of the above sample solution in 100 ml with diluent II and mix.

Chromatographic Parameters

Column	**Waters Symmetry C-18, 250 x 4.6mm, 5µm.**
Flow rate	1.0 ml/min
Wavelength	215 nm
Injection volume	10µl
Column oven temperature	30°C
Sample oven temperature	20°C
Run time	10 minutes

PROCEDURE

The column was equilibrated with mobile phase for 30to45 minutes and blank, standard solution (six replicate), sample solution (duplicate injection) were injected in the following sequence-

Sr. No.	**Sample Name**	**No. of Injections**
1.	Blank	1
2.	Standard solution	6
3.	Sample solution	2
4.	Bracketing Standard	1

Calculation

$$\%\text{Assay} = \frac{AT}{AS} \times \frac{WS}{25} \times \frac{5}{100} \times \frac{250}{WT} \times \frac{100}{5} \times \frac{AW}{LC} \times \frac{P}{100} \times 100$$

Where,

AT: The average area of nefopam hydrochloride in the sample solution
AS: The average area of nefopam hydrochloride in standard solution
WS: Weight of nefopam hydrochloride working standard in mg.
WT: Weight of test sample in mg.
AW: The average weight of nefopam hydrochloride tablets is in mg.
LC: Label claim of nefopam hydrochloride in mg.
P: the potency of nefopam hydrochloride working standard on an as-is basis.

METHOD VALIDATION

The objective of the validation of the analytical procedure is to demonstrate that it is suitable for its intended purpose. Guidelines from USP, ICH, US-FDA, etc can provide a framework for validation of pharmaceutical methods. Results from the method validation can be considered to judge its quality, reliability as well consistency about analytical results.

1) System Suitability

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations, and samples to be analyzed

constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated(7).

Acceptance Criteria

- The %RSD of area for nefopam hydrochloride peak in standard solution for six replicate injections should be not more than 2.0
- The tailing factor of nefopam hydrochloride peak in standard solution should be not more than 2.0
- The Theoretical plates of nefopam hydrochloride peak in standard solution should not be less than 2000.

2) Specificity and Selectivity

Specificity is the ability to assess unequivocally the analyte in the 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(s)(7).

Acceptance Criteria

- No peak should be eluting at the retention time of nefopam hydrochloride due to benzhydrol, benzamide, o-benzoylbenzoic acid, blank, and placebo solution.
- Peak purity should be observed for nefopam hydrochloride peak in standard solution and spiked sample solution.

3) 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 (amount) of analyte in the sample. Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. If there is a linear relationship, test results should be evaluated by appropriate statistical methods, for example, by calculation of a regression line by the method of least squares.

The correlation coefficient, y-intercept, slope of the regression line, and residual sum of squares should be estimated. In addition, an analysis of the deviation of the actual data points from the regression line may be evaluated. For the establishment of linearity, a minimum of 5 concentrations is recommended(7).

Acceptance Criteria

- The correlation coefficient ‘r’ should not be less than 0.999.

4) Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy should be established across the specified range of the analytical procedure.

Accuracy should be assessed using a minimum of 9 determinations over at least 3 concentration levels covering the specified range. Accuracy should be reported as percent recovery of analyte in the sample or as the difference between the mean and the accepted true value together with the confidence intervals (7).

Acceptance criteria

- Recovery of nefopam hydrochloride at each level should be within 98.0% and 102.0% with a %RSD of not more than 2.0.
- The overall average percent recovery should be within 98.0% and 102.0% with a %RSD of not more than 2.0.

5) 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 deviation, or coefficient of variation of a series of measurements.

1) **Repeatability:** Repeatability or intra-assay precision expresses the precision under the same operating conditions over a short interval of time. Repeatability should be assessed using:
    a. A minimum of 9 determinations covering the specified range for the procedure (e.g., 3 concentrations/3 replicates each); or
    b. A minimum of 6 determinations at 100% of the test concentration.
2) **Intermediate precision:** Intermediate precision expresses within-laboratories variations such as different days, different analysts, different equipment. The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used.
3) **Reproducibility:** Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology). Reproducibility is assessed using an inter-laboratory trial.

Standard deviation, relative standard deviation (coefficient of variation), and confidence interval should be reported for each type of precision investigated.

Acceptance Criteria

- System suitability criteria should be passing.
- % RSD for %assay of six sample preparations should be not more than 2.0
- Overall % RSD for %assay of twelve sample preparations (six of method precision and six of intermediate precision) should be not more than 2.0

6) Filter study

Filter retention studies are a comparison of filtered to unfiltered solutions during a method validation to determine whether the filter being used retains any active compounds or contributes unknown compounds to the analysis. Blank, sample, and standard solutions are analyzed with and without filtration. Comparisons are made in the recovery and appearance of chromatograms.

Acceptance criteria

- The absolute difference between % assay obtained from centrifuged sample solution and filtered sample solution should be not more than 2.0

7) Solution stability

The stability of standards and samples is established under normal benchtop conditions, normal storage conditions, and sometimes in the instrument (e.g., an HPLC autosampler) to determine if special storage conditions are necessary, for instance, refrigeration or protection from light.

Stability is determined by comparing the response and impurity profile from aged standards or samples to that of a freshly prepared standard and to its response from earlier time points.

Acceptance Criteria

- **For standard solution**: Cumulative % RSD at each time interval along with the initial six injections of standard solution should not be more than 2.0
- **For sample solution:** The absolute difference in % assay of sample solution should not be more than 2.0 at each time interval.

8) Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of analysis concerning deliberate variations in method parameters[5].

Acceptance Criteria

- System suitability criteria should be passing.
- The overall % RSD for % assay of one sample of robustness and six samples of method precision solution should be not more than 2.0

RESULTS AND DISCUSSION

The optimized method was validated as per the above guidelines. The retention time of nefopam hydrochloride was 5.313 minutes. Data of the validation exercise is given below.

1. **System suitability:** Since all the acceptance criteria have been attained, the system is found to be suitable. Details of same are available in Table 1.

Table 1: System Suitability parameters of nefopam hydrochloride

Sr. No.	Number of Theoretical Plates	Tailing Factor	Peak area of nefopam hydrochloride
1	9053	1.14	7455229
2	8889	1.15	7451016
3	9238	1.14	7433567
4	9056	1.13	7432472
5	9186	1.12	7443548
6	9301	1.15	7424326
Mean			**7440026**
SD			**11912.9122**
%RSD			**0.16**

2. **Specificity & selectivity:** Figures 2 A to 2 C are chromatograms of study for specificity and selectivity while Table 2 gives the values of specificity parameters.

   It was observed that no peak was eluted at the retention time of nefopam hydrochloride due to benzhydrol, benzamide, o-benzoylbenzoic acid (reported impurities of nefopam hydrochloride), blank and placebo solution. Peak purity is passing for nefopam hydrochloride peak in standard solution and sample solution.Hence it has been concluded that the method is selective for the assay of nefopam hydrochloride in the formulation.

Table No.2: Specificity (Selectivity) parameters of nefopam hydrochloride

Sample	RT of Nefopam hydrochloride	Peak purity index
Blank	ND	NA
Placebo	ND	NA
Standard	5.413	1.0000
Sample (Unspiked)	5.407	1.0000
Sample (Spiked)	5.407	1.0000
Benzamide	ND	NA
Benzhydrol	ND	NA
Benzoylbenzoic	ND	NA

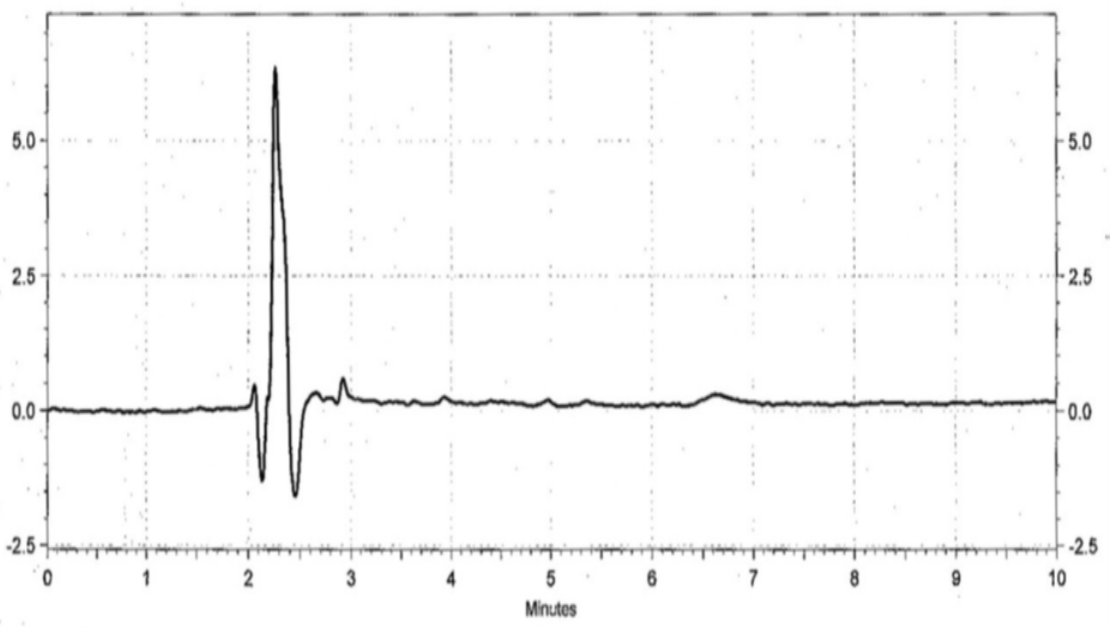


Fig.2A: Chromatogram of blank

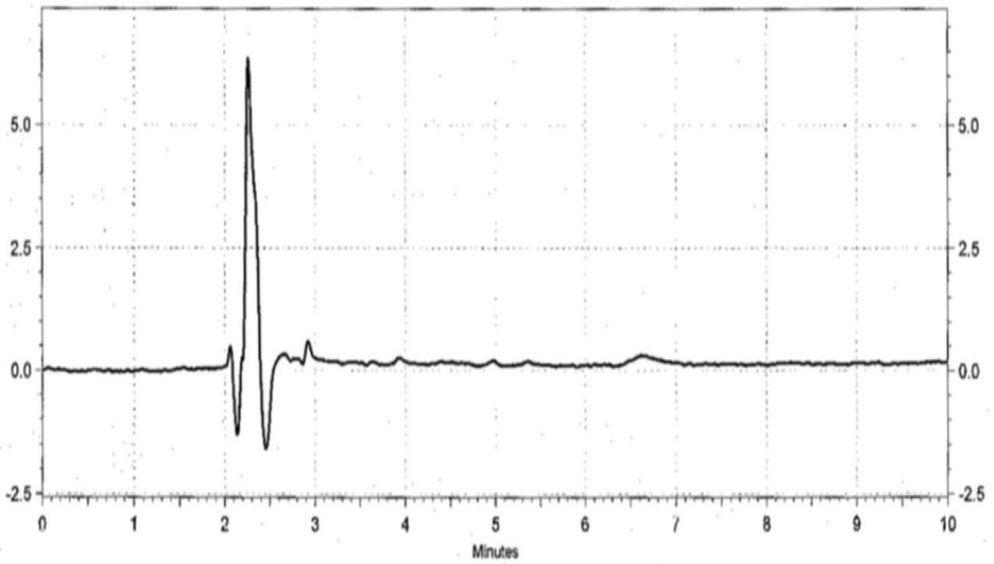
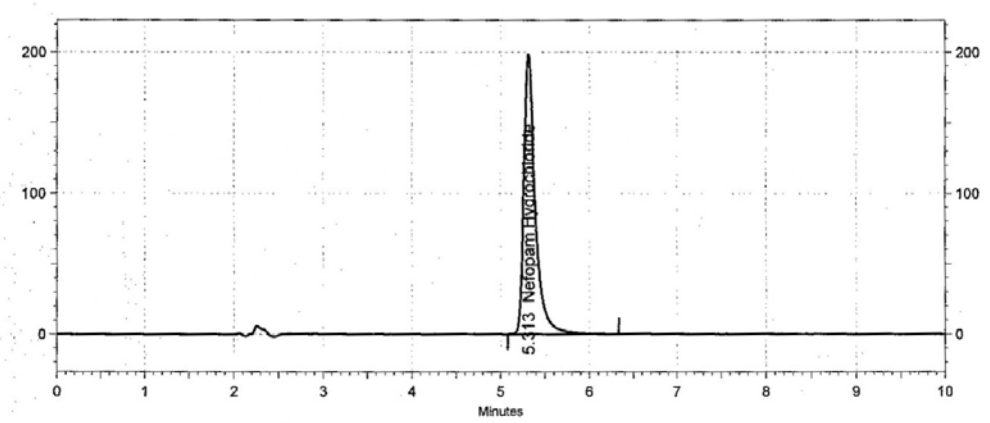


Fig.2B: Chromatogram of placebo



DAD: Signal A,
215 nm/Bw:4 nm
Results

Name	Retention Time	Area	Area Percent	Theoretical plates (USP)	Asymmetry	Peak purity
Nefopam Hydrochloride	5.313	3678958	100.000	9844	1.753	1.000000

Fig.2C: Standard chromatogram ofnefopam hydrochloride

3. Linearity and Range

The correlation coefficient (r) value is within the acceptance criteria (Table 3). Also, the detector response of nefopam hydrochloride is linear in the concentration range of 16 ppm to 120 ppm (Figure 3).

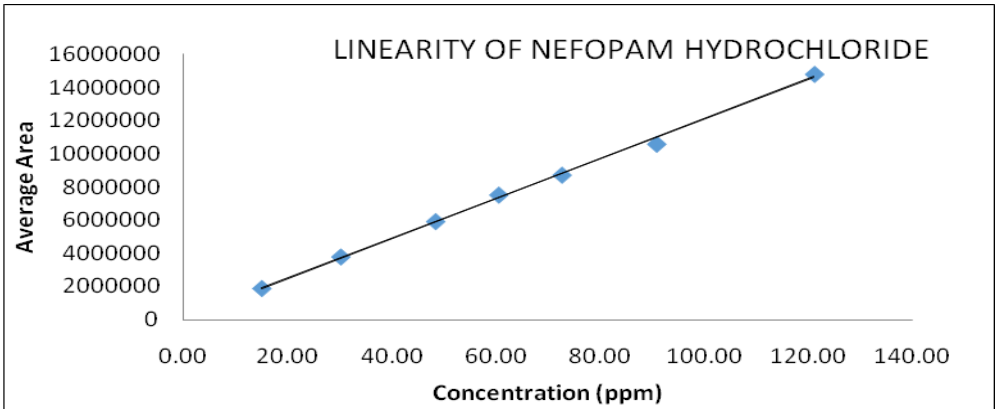


Fig. 3: Linearity plot for Nefopam Hydrochloride

Table 3: Linearity parameters of Nefopam Hydrochloride

Concentration level (%)	Vol. Added from stock solution (ml)	Diluted to (ml)	Concentration (ppm)	Average peak area
25	2.5	200	15.13	1901677
50	2.5	100	30.27	3804749
80	4.0	100	48.43	5934529
100	5.0	100	60.54	7531156
120	6.0	100	72.65	8733333
150	7.5	100	90.81	10600349
200	5.0	50	121.08	14810373

Correlation coefficient (r)	0.999
Slope	119512.193
Intercept	123009.703
%Y-Intercept	1.6

4. Accuracy

Accuracy results at various levels of concentration are summarized in Table No. 4. For accuracy studies, the limit for percent mean recovery is 98%-102%. From the results, it can be seen that the percent mean recovery is 100.4% which is within the limit, hence the method is accurate.

Table 4: Accuracy Parameters of Nefopam Hydrochloride

Level (%)	Weight of Placebo (mg)	Actual amount of drug added (mg)	Amount recovered (mg)	% Recovery	Average % Recovery	SD	% RSD
25	1852.4	75.52	76.9	101.8	101.7	0.0577	0.06
	1848.9	75.22	76.5	101.7			
	1861.0	74.82	76.1	101.7			
50	1842.0	149.45	149.4	100.0	100.5	0.8963	0.89
	1852.4	149.65	149.5	99.9			
	1849.7	149.75	152.0	101.5			
100	1852.0	299.10	296.8	99.2	99.2	0.0577	0.06
	1848.1	298.70	296.5	99.3			
	1839.8	298.60	296.3	99.2			
150	1845.2	448.35	456.6	101.8	101.4	0.4041	0.40
	1849.6	447.55	452.0	101.0			
	1852.4	447.95	453.7	101.3			
200	1839.8	597.10	593.3	99.4	99.4	0.3000	0.30
	1842.4	597.30	595.4	99.7			
	1845.4	596.90	591.7	99.1			
Overall Average % Recovery				**100.4**			
Overall SD				**1.1128**			
Overall % RSD				**1.11**			

5. Precision

The % RSD values were found to be within the limit that is less than 2%. The results are summarized in Table 5.The mean assay percentage results are summarized in Table 5A and 5B and are found to be within limits. Table 5C to 5E gives data for precision studies and it is observed that % RSD complies with the limits as per ICH guidelines.

Table 5: System Precision

Sr. No.	Peak area of Nefopam Hydrochloride
1	7455229
2	7451016
3	7433567
4	7432472
5	7443548
6	7424326
Mean	**7440026**
SD	**11912.9122**
%RSD	**0.16**

Table 5A: Method Precision (System Suitability)

Sr. No.	Theoretical Plates	Tailing Factor	Peak area of Nefopam Hydrochloride
1	9053	1.14	7455229
2	8889	1.15	7451016
3	9238	1.14	7433567
4	9056	1.13	7432472
5	9186	1.12	7443548
6	9301	1.15	7424326
Mean			**7440026**
SD			**11912.9122**
%RSD			**0.16**

Table No. 5B: Method Precision

Sr. No.	Average peak area	% Assay
1	7621168	101.3
2	7636471	101.3
3	7576017	100.5
4	7545032	99.8
5	7518853	99.4
6	7514178	99.8
Mean %assay		**100.4**
SD		**0.8167**
%RSD		**0.81**

Table No. 5C: Intermediate Precision (System Suitability)

Sr. No.	Theoretical Plates	Tailing Factor	Peak area of Nefopam Hydrochloride
1	2671	1.85	7281985
2	2664	1.87	7063600
3	2679	1.87	7162498
4	2637	1.89	7176046

5	2676	1.87	7240439
6	2724	1.91	7278181
Mean			**7200458**
SD			**83729.3453**
%RSD			**1.16**

Table No. 5D: Intermediate Precision

Sr. No.	Average peak area	% Assay
1	7531384	101.8
2	7362678	100.0
3	7415151	100.6
4	7503082	101.6
5	7343859	99.8
6	7312319	99.3
Mean %assay		**101.5**
SD		**1.0088**
%RSD		**1.00**

Table No. 5E: Intermediate Precision (Overall %RSD)

Parameter	Intermediate Precision	Method Precision
1	101.8	101.3
2	100.0	101.3
3	100.6	100.5
4	101.6	99.8
5	99.8	99.4
6	99.3	99.8
Mean	**100.5**	**100.4**
SD	**1.0088**	**0.8167**
%RSD	**1.00**	**0.81**
Overall Mean	**100.4**	
Overall SD	**0.8794**	
Overall %RSD	**0.88**	
Analyst Name	Analyst-I	Analyst-II

6. Filter study

From the data obtained (Table 6), it is concluded that 0.45µm nylon filter, 0.45µm PTFE filter, and 0.45µm PVDF filter are suitable filters for filtering the sample solution of nefopam hydrochloride.

Table 6: Filter Study

Sr. No.	Filter used	% Assay	Absolute difference
1	Centrifuge	99.6	NA
2	0.45µm Nylon Filter	100.4	-0.80
3	0.45µm PTFE Filter	101.2	-1.60
4	0.45µm PVDF Filter	100.2	-0.60

7. Solution stability

Data of stability of nefopam hydrochloride in solution form is summarized in Tables 7A and 7B. It is seen that nefopam hydrochloride standard solution is stable up to 50 hours at room temperature while the sample solution is stable up to 49 hours.

Table 7A: Solution stability (standard solution at room temperature)

Time (Hours)	Peak Area	Cumulative Mean	Cumulative SD	Cumulative %RSD
0	7434141	NA	NA	NA
1	7430904	7433679	12068.5689	0.16
4	7456756	7437372	14738.1686	0.20
11	7453202	7436864	14001.9446	0.19
17	7413454	7431186	14327.9842	0.19
23	7503582	7444061	28861.9333	0.39
29	7476624	7440210	20049.4001	0.27
35	7503004	7443979	28663.4137	0.39
41	7514606	7445636	32696.9521	0.44
47	7537557	7448915	40889.8735	0.55
50	7536678	7448789	40572.4047	0.54

Table No. 7B: Solution stability (sample solution at room temperature)

Time (In hours)	Peak Area	%Assay	Absolute Difference
0	7596718	100.9	NA
3	7563340	100.5	0.40
10	7588455	100.8	0.10
16	7617936	101.2	-0.30
22	7592730	100.9	0.00
28	7595981	100.9	0.00
34	7629881	101.4	-0.50
40	7648511	101.6	-0.70
46	7677789	102.0	-1.10
49	7699021	102.3	-1.40

8. Robustness

By analyzing robustness, resultant% RSD values were found to be within the limit that is less than 2%, thus the developed method was confirmed to be robust. The results are summarized in Table 8.

Table 8: Robustness (System Suitability)

Sr. No.	Parameter	%Relative Standard Deviation	Number of Theoretical Plates	Tailing Factor	Overall %RSD
1.0	Plus Flow rate (1.1ml/min)	0.19	8574	1.18	0.75
2.0	Minus Flow rate (0.9ml/min)	0.15	10226	1.14	0.88

3.0	Plus pH of mobile phase buffer (pH-2.9)	0.90	9469	1.16	0.84
4.0	Minus pH of mobile phase buffer (pH-2.5)	0.18	9628	1.14	0.92
Mobile Phase Composition					
5.1	Buffer: ACN: Methanol (50.5:30:19.5)	0.20	8736	1.19	0.86
5.2	Buffer: ACN: Methanol (59.5:30:10.5)	0.18	10325	1.14	0.90
6.0	Plus Wavelength (218 nm)	0.21	10660	1.14	0.90
7.0	Minus Wavelength (212nm)	0.29	10359	1.13	0.77
8.0	Plus Column oven Temp (35ºC)	0.43	10966	1.12	0.95
9.0	Minus Column oven Temp (25ºC)	0.21	11482	1.14	0.80
10.0	Plus Extraction time	0.41	11217	1.14	0.88
11.0	Minus Extraction time	0.28	11334	1.14	0.94

CONCLUSION

The RP-HPLC method development was found to be simple, precise, rapid, accurate for the quantification of nefopam hydrochloride in its tablet dosage form.

The method was reliable in terms of system suitability, linearity, precision, accuracy and recovery, robustness, and assay. The data was supported by filter study and solution stability.

All the verification parameters were within the range according to ICH Q2A (R1) guidelines. Hence, the authors conclude that the proposed RP-HPLC method can be used for routine analysis of nefopam hydrochloride in the pharmaceutical industry.

ABBREVIATIONS

RP-HPLC: Reversed-Phase High-Performance Liquid Chromatography; ICH: International Council for Harmonization; UV-VIS: Ultraviolet-visible SST: System suitability, spectrophotometry; D: Standard deviation; %RSD: Percentage relative standard deviation; NMT: Not more than.

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