A NOVEL STABILITY INDICATING METHOD FOR NEFOPAM IN PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC

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ABSTRACT

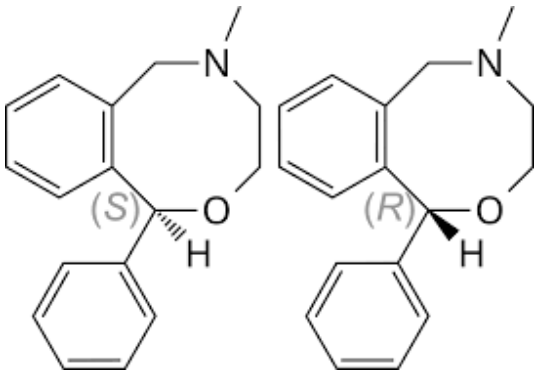
A stability indicating reverse phase high performance liquid chromatography. (RP-HPLC) method was developed and validation for the determination of Nefopam in pharmaceutical dosage forms. The method utilized a Agilent, kromasil C18[4.6*250mm] with a mobile phase consisting of[Methanol:Water(50:50%v/v),water:acetonitril(60:40%v/v),methanol:0.1%OPA(50:50%v/v),acetonitrile:0.1%OPA(50:50%v/v),60%0.01NKh2po4:40%acetonitrile]. The flow rate was set to 1ml/min with the response Nefopam was detected at [260nm]. Retention time of Nefopam was found to be 2.338 min. %RSD of the Nefopam were and found to be 0.9%. %RSD of Method precision of Nefopam was found to be 0.2. %Recovery was obtained as 99.68% for Nefopam. LOD, LOQ values obtained from regression equation of Nefopam were 0.03, 0.08. Regression equation of Nefopam is y = 19879x + 2947. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Keywords: Nefopam, RP-HPLC, Validation, Accuracy, Robustness.

INTRODUCTION

Nefopam is a non-opioid analgesic (5-methyl-1-phenyl-3,4,5,6-tetrahydro-1H-2,5-benzoxazocine hydrochloride) used for moderate to severe pain, especially post-surgical pain.

Structure:



Indication: Nefopam is a painkiller. It treats moderate pain, for example after an operation or a serious injury, dental pain, joint pain and muscle pain, or pain from cancer. You can also take nefopam for other types of long-term pain when weaker painkillers no longer work. Nefopam is available on prescription only.

Mechanism of action:

Nefopam is a non-opioid, centrally-acting analgesic. The mechanism of action is not well understood. It is known to inhibit the re-uptake of neurotransmitters including serotonin, noradrenaline and dopamine. Additionally, it blocks sodium and calcium channels in the central nervous system.

MATERIALS AND METHODS

Materials:

Nefopam pure drug (API), Nefopam formulation (**ACTIZA**), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals andsolvents are from Rankem.

Instruments:

- Electronics Balance-Denver
- p^H meter -BVK enterprises, India
- Ultrasonicator-BVK enterprises
- WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software.
- UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbance of Nefopam solution.

Methods:

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50

Preparation of Standard stock solutions: Accurately weighed 10mg of Nefopam is transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (200mg of Nefopam)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (20mg of Nefopam).

Preparation of Sample stock solutions: 10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 50ml volumetric

flask, 20ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (400mg of Nefopam)

Preparation of Sample working solutions (100% solution): 0.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (20mg of Nefopam)

Preparation of buffer:

0.01N KH_2PO_4 Buffer: Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 4.8 with dil. Orthophosphoric acid solution.

0.1%OPA Buffer: 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade

water.

Validation:

System suitability parameters:

The system suitability parameters were determined by preparing standard solution of Nefopam (30ppm) and the solution were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.The % RSD for the area of six standard injections results should not be more than 2%.

Specificity: Checking of the interference in the optimized method. 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.

Precision:

Preparation of Standard stock solutions: Accurately weighed 10mg of Nefopam is transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (200mg of Nefopam)

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Preparation of Sample working solutions (100% solution): 0.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (20mg of Nefopam)

Linearity:

Preparation of Standard stock solutions: Accurately weighed 10mg of Nefopam is transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (400µg/ml of Nefopam)

25% Standard solution: 0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (5µg/ml of Nefopam)

50% Standard solution: 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (10µg/ml of Nefopam)

75% Standard solution: 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (15µg/ml of Nefopam)

100% Standard solution: 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (20mg of Nefopam)

125% Standard solution: 1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (25µg/ml of Nefopam)

150% Standard solution: 1.5ml each from two standard stock solutions was pipettede out and made up to 10ml (20µg/ml of Nefopam)

Accuracy:

Preparation of Standard stock solutions: Accurately weighed 10mg of Nefopam is transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (200mg of Nefopam)

Preparation of 50% Spiked Solution: 0.25ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 0.75ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Nefopam, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Nefopam, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

Degradation studies:

Oxidation:

To 1 ml of stock solution of Nefopam, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 20µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies:

To 1 ml of stock solution Nefopam, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c. For HPLC study, the resultant solution was diluted to obtain 20µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:

To 1 ml of stock solution Nefopam, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0c. For HPLC study, the resultant solution was diluted to obtain 20µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies:

The standard drug solution was placed in oven at 105°C for 6h to study dry heat degradation. For HPLC study, the resultant solution was diluted to obtain 20µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Photo Stability studies:

The photochemical stability of the drug was also studied by exposing the 400µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 20µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies:

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to obtain 20µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

RESULTS AND DISCUSSION

System suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines

Table:1. System suitability parameters for Nefopam

S no	Nefopam		
Inj	RT(min)	USP Plate Count	Tailing
1	2.349	6419	1.31
2	2.349	6419	1.31
3	2.354	6694	1.30
4	2.364	6610	1.30
5	2.373	6400	1.30
6	2.379	6528	1.30

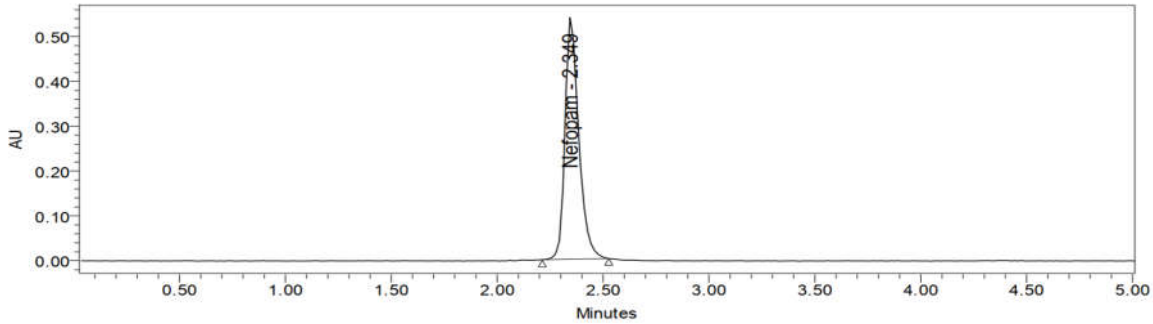

Figure No:1. System suitability Chromatogram

Linearity:

Table:2. Linearity table for Nefopam.

Nefopam	
Conc (µg/mL)	**Peak area**
0	0
5	100816
10	202292
15	304233
20	405335
25	504132
30	591138

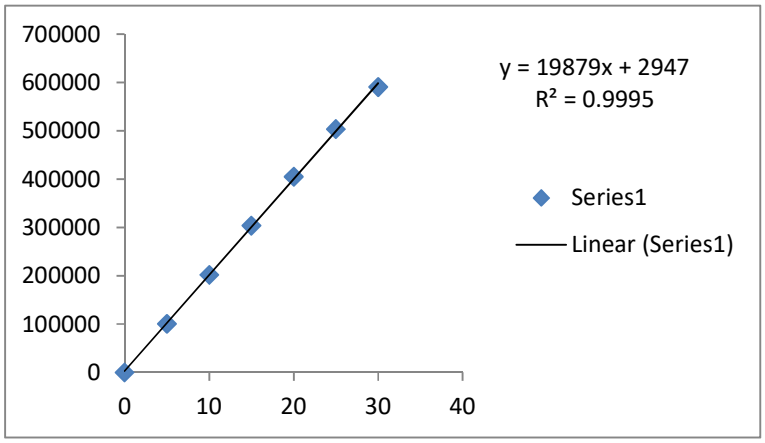

Figure No:2. Calibration curve of Nefopam

Precision:

System Precision:

Table:3. System precision table of Nefopam

S. No	Area of Nefopam
1.	403156
2.	398653

3.	399865
4.	408543
5.	399625
6.	403964
Mean	402301
S.D	3711.1
%RSD	0.9

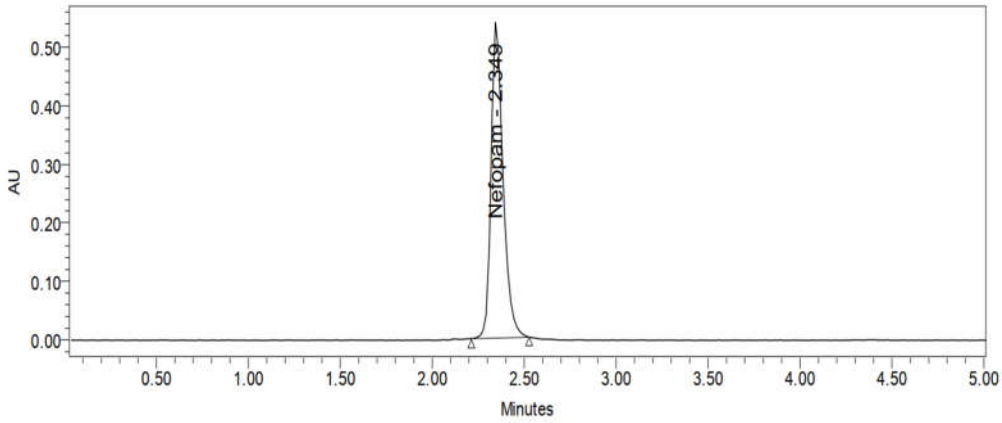

Figure No:3. System precision chromatogram

Method precision:

Table:4. Method precision table of Nefopam.

S. No	Area of Nefopam
1.	401234
2.	401687
3.	402354
4.	401021
5.	399623
6.	401902

Mean	401304
S.D	950.5
%RSD	0.2

Accuracy:

Table:5. Accuracy table of Nefopam

% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	Mean %Recovery
50%	10	9.97	99.65	99.68%
	10	9.97	99.75	
	10	9.99	99.91	
100%	20	19.97	99.83	
	20	20.02	100.12	
	20	19.93	99.65	
150%	30	29.84	99.45	
	30	29.79	99.29	
	30	29.84	99.46	

Table:6. Sensitivity table of Nefopam

Molecule	**LOD**	**LOQ**
Nefopam	0.03	0.08

Robustness:

Table:7. Robustness data for Nefopam

S.no	Condition	%RSD of Nefopam
1	Flow rate (-) 0.9ml/min	0.3
2	Flow rate (+) 1.1ml/min	0.8
3	Mobile phase (-) 65B:35A	0.1
4	Mobile phase (+) 55B:45A	0.4
5	Temperature (-) 27°C	0.6
6	Temperature (+) 33°C	0.6

SUMMARY AND CONCLUSION

Parameters	**Nefopam**	**LIMIT**
Linearity Range(µg/ml)	5-30µg/ml	R< 1
Regressioncoefficient	0.999	
Slope(m)	19879	
Intercept(c)	2947	
Regression equation (Y=mx+c)	y =19879x + 2947	
Assay (% mean assay)	99.68%	90-110%
Specificity	Specific	No interference of any peak
System precision %RSD	0.9	NMT 2.0%
Method precision %RSD	0.2	NMT 2.0%

Accuracy%recovery		99.68%	98-102%
LOD		0.03	NMT 3
LOQ		0.08	NMT 10
Robustness	**FM**	0.3	%RSD NMT 2.0
	FP	0.8	
	MM	0.1	
	MP	0.4	
	TM	0.6	
	TP	0.6	

Conclusion

An simple, precise, accurate sensitive and specific RP-HPLC method for the determination of Nefopam in pharmaceutical dosage form. Retention time of Nefopam was found to be 2.338 min. %RSD of the Nefopam were and found to be 0.9%. %RSD of Method precision of Nefopam was found to be 0.2. %Recovery was obtained as 99.68% for Nefopam. LOD, LOQ values obtained from regression equation of Nefopam were 0.03, 0.08. Regression equation of Nefopam is y = 19879x + 2947. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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