Development and validation of reverse phase high performance liquid chromatographic method for quantitative estimation of nefopam in tablet dosage form.

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Abstract

A simple, precise and rapid Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method has been developed for quantitative determination of Nefopam in tablet dosage form. The chromatographic separation was achieved on Inert sustain swift C18 column (250 mm×4.6 mm id,5µ). The mobile phase is water with 0.1% triethylamine with pH adjusted to 3.0 using dilute OPA and acetonitrile in the ratio of 45: 55v/v in an isocratic mode with column temperature 40°C was used. The detection was carried out at 229 nm, 10µl injection volume was selected with the flow rate 1.5 ml/min. The retention time was found to be 5.1 min.Themethod was linear over range of 50-150µg/ml. The regression coefficient obtained was 0.999.Water: acetonitrile was used as a diluent. The method was validated as per ICH guidelines and study reveals that the developed method is specific, rapid, reliable and reproducible hence it can be applied for routine quality control analysis of Nefopam in tablet dosage form.

Keywords: Nefopam, HPLC, Validation, Formulations

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Introduction

Nefopam hydrochloride, 5-methyl-1-phenyl-1,3,4,6-tetrahydro-2,5-benzoxazocine, is a cyclic analogue of diphenhydramine and an analgesic which is active both orally and parenterally. Nefopam is a centrally acting analgesic which does not compromise ventilation and is minimally sedating. Its efficacy relies on medullar and/ or supramedullar mechanisms. Its fast onset of action is an advantage, especially in the treatment of postoperative pain (Figure 1) [1].

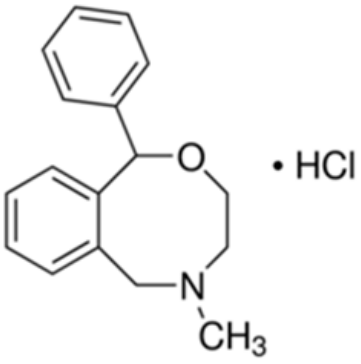

Figure1: *Structure of Nefopam.*

Literature survey reveals that very few chromatographic methods were developed for the estimation of Nefopam in tablet dosage forms using triethylammonium phosphate buffer and gradient system with low system suitability Parameter and diode-array detection system for HPLC, LC-MS systems also the methods are mostly a consolidated Pharmcokinetics study. Hence, attempts were made to develop a simple, rapid, precise and accurate reverse phase chromatographic method to estimate Nefopam in Tablet dosage form. This method has been successfully used for Quality control analysis of Nefopamtablet formulation. The proposed method was optimized and validated according to International Conference on Harmonization (ICH) guidelines. The objective is to give an overview of the mechanism of Reversed Phase High Performance Liquid Chromatography (RP-HPLC) of Nefopam and explain the basis of the retention mechanism and to achieve high speed separation without any loss of reproducibility.

Materials and Methods

Chemicals and reagents

An analytically pure Nefopam working standard was procured from Central Drug Testing Laboratory, Mumbai with defined potency [99.98% as is basis]. NEFOSAR (30 mg)Nefopamtablets were received from In-house R and D laboratory of AbottLife SciencesMumbai. HPLC Grade Acetonitrile from Himedia, triethylamine AR Grade from Spectrochem and Water-milli-Q Grade were used.

Instrumentation

Perkin Elmer UV/ VIS Spectrophotometer Lambda 25 connected to Perkin Elmer UV Win Lab softwarewas used for all the spectrophotometric measurements. Thermo Scientific DionexChromeleon Chromatography Data System Version 7.2.6 with LC instrument control was used for chromatography.

The column used was InertsustaineC18 column (250 mm × 4.6 mm × 5 µ) from LCGC.

Selection of solvent (diluent)

On the basis of Molecular structure and chemical nature of Nefopam, Water was selected as diluent for preparation of standard and sample solutions.

Selection of wavelength

About 10.0 mg of Nefopam was transferred to the 100ml volumetric flask and the volume was made up to the mark with diluent and aliquot portions of standard stock solutions of Nefopam were diluted appropriately with diluent to obtain concentration of 10µg/ml of drug. The solution was scanned in the range of 200 nm to 400 nm. The absorbance maximum of Nefopam was found at 229 nm (Figure 2) [2].

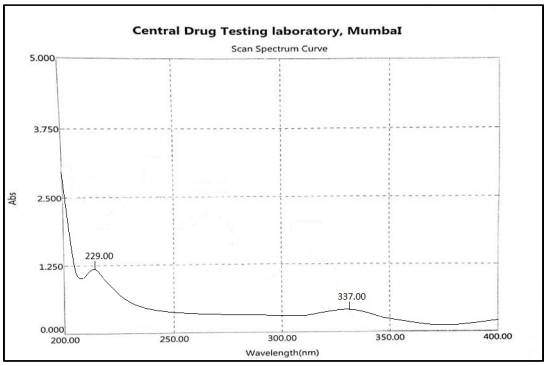

Figure2: *NefopamUV spectrum.*

Preparation of standard drug solution: Accurately weighted about 10 mg of Nefopam standard was transferred in a 100ml volumetric flask and dissolved by sonication in sufficient diluent then volume made with diluent (100 µg/ ml). Then 5 ml from above stock solution was diluted up to 10 ml with the same diluent (50 µg/ ml).

Preparation of sample solution: Twenty Tablets of Nefosar (30 mg) were weighed and average weight was calculated. The tablet contents were crushed to fine powder. An accurately weighed quantity of tablet powder equivalent to 10 mg of Nefopam was dissolved in diluent and sonicated for 15 min. Final dilution was made up to100ml with diluent (100 µg/ ml). Then 5 ml from above stock solution was diluted up to 10 ml with the same diluent (50 µg/ ml).

Method optimization

According to chemical structure of the Nefopam an Endcapped column such as Inertsustaine C18 column was selected for the retention of Nefopam. Initial trials were started with Water and Acetonitrile of different proportionswith different C18 column, An Inertsustaine column was found to be suitable when compared to other C18 columns. Good peak shape and better SST parameters were obtained after the addition of 0.1% triethylamine with mobile phase consisting of Water and Acetonitrile in the ratio of 45:55 v/v. Flow rate was kept at 1.5 ml/min and UV detection wavelength of 229 nm and column oven temperature maintained at 30°C. Figures3 and 4

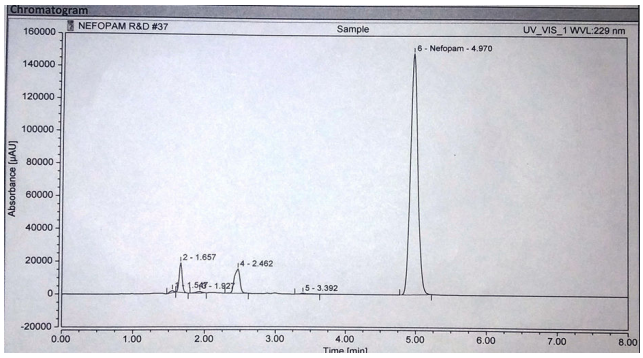

Figure3*: Chromatogram of Nefopam standard solution.*

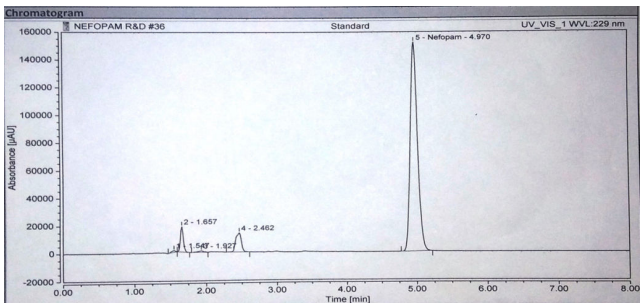

Figure4: *Chromatogram of Nefopam sample solution.*

Validation of method

Validation of developed method was done as per ICH Q2 (R1) 22 guidelines with respect to various parameters such as linearity, accuracy, precision and robustness. Standard solution of Nefopam was used for comparison of results.

Appropriate aliquots from standard Nefopam stock solutions were prepared to obtain concentrations of 50- 150µg/ml. The linear calibration plot was constructed by analysing the concentrations over the selected range. The response for the drug was linear in the concentration range between 50-150 µg/ml. The linearity was observed in the expected concentration range, demonstrating its suitability for analysis. (Figure 5 and Table 1) [4].

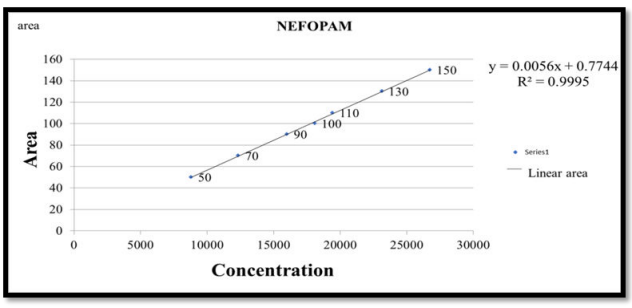

Figure5: *Linearity Curve of Nefopam.*

Table1: *Linearity of Nefopam.*

Linearity level	Concentration (µg/ml)	Area
1	50	8808

2	70	12348
3	90	15997
4	100	18095
5	110	19435
6	130	23135
7	150	26741

Standard Addition method

Good recovery of the spiked drug was obtained at each added concentration, indicating that the method was accurate. A known amount of Nefopam (110, 120, and 130%) of standard solution was added to the pre analysed solution of formulation. This solution was analysed as previously described. The assay was repeated over 3 injections of each concentration to obtain data. The resultant % RSD for this study was found to be < 2.0 % with a corresponding percentage recovery value as shown in Table 2.

Table2: *Accuracy by Standard addition method (% Recovery study.*

Wt. of std spiked(mg)	Wt of sample Eq to 100mg	% Concentration	*Peak area of samples	Peak area of standard	mg found	% Drug Recovery
10.6	30.4	110%			10.331	100.31
		110%	19453	16108		
		110%				
20.8	30.3	120%			20.548	100.56
		120%	23122			
		120%				
30.4	30.7	130%			30.115	100.47
		130%	26704			
		130%				
Mean			(NLT 98% and NMT 102%)	100.45		
± SD					0.103	
% RSD			(NMT 2%)		0.103	

Precision

Precision of System (Repeatability) was ascertained by six replicate analysis of 50 µg/ml Standard solution of Nefopam. Precision of method was ascertained by six replicate analyses of homogeneous sample of tablet powder at concentration 50µg/ml. The Intermediate precision (Ruggedness) was studied by injecting freshly prepared working standard solution of Nefopam on two different days (interday) and on same day but at three different time (Intraday) (Tables 3, 4, 5 and 6) [5].

Table3: *System precision.*

Injection No.	Area at 238 nm	Limit
1	17815	NMT 2.0 %
2	17822	
3	17776	
4	17805	
5	17756	
6	17725	
Mean	17783.11	
± S.D.	34.4995	
% RS.D.	0.1938	

Table4: *Method precision (Repeatability).*

Injection No.	Area at 229 nm	Limit
1	17314	NMT 2.0%
2	17295	
3	17285	
4	17285	
5	17280	
6	17269	
Mean	17288.02	
± SD	14.0021	
% RSD	0.081	

Table5: *Intermediate Precision (Intraday Precision).*

Sr. No.	Analyst A (Day 1)	Analyst B (Day 2)	Analyst C (Day 3)
1	101.54	98.61	98.34
2	99.24	99.49	99.33
3	98.57	100.73	99.46
4	98.34	99.63	100.53
5	100.35	100.28	99.67
6	98.73	99.3	98.71
Average	99.46	99.67	99.34
S.D.	1.1362	0.6819	0.6996
%R.S.D.	1.1424	0.6842	0.7042

Table6: *Intermediate Precision (Intraday Precision).*

Sr. No.	Morning % assay	Aftertnoon % assay	Evening % assay
1	99.76	100.64	100.1
2	99.46	99.58	99.39

3	100.35	99.31	99.74
4	98.71	99.82	99.65
5	100.29	99.73	100.56
Mean	99.71	99.82	99.89
SD	0.6018	0.4468	0.4057
% RSD	0.6035	0.4476	0.4062

Specificity

Specificity of the method was established to ascertain how accurately and specifically the analyte of interest are estimated in presence of excipients. The results of the specificity done by injecting Blank (Mobile phase) and Placebo solution, showed there was no interference and co-elution of any other peaks with the retention of Nefopam. The peak purity of Nefopam in Tablet dosage forms found within the limit which proved that there was no interference of the blank peaks and excipient peaks at the retention time of Nefopam (Figures 6 and 7) [6].

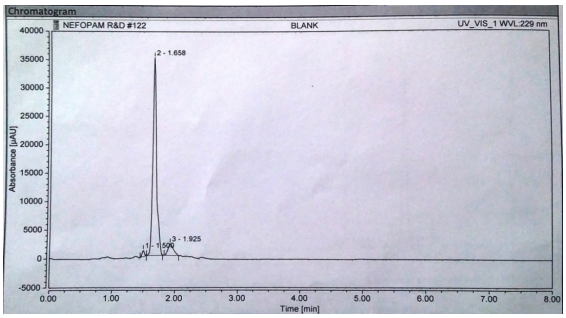

Figure6: *Chromatogram of blank solution.*

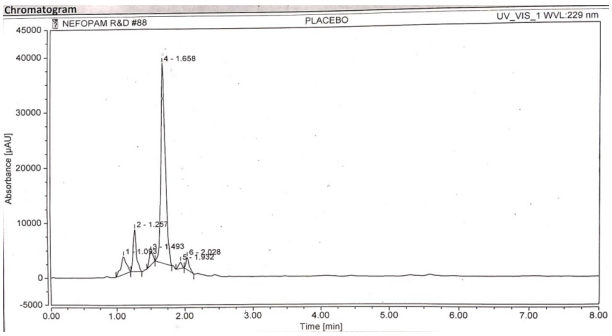

Figure7: *Chromatogram of Placebo solution.*

Robustness: The Robustness of the method was established by deliberate change in detection wavelength by ± 2 nm, change in the temperature by ± 2°C and flow rate by ± 0.2 ml in the estimation of Tablet. The reproducible results were obtained which proves that method is robust as shown in Table 7.

Table7: *Robustness.*

Parameter	Parameter (±)	% Estimation	Mean	S.D.	% R.S.D.
Wavelength (± 2 nm)	227	97.04	97.97	0.6594	0.673
	229	98.34			
	231	98.52			
Temperature (± 3°C)	38°C	97.04	97.63	0.6388	0.6543
	40°C	97.34			
	43°C	98.52			
Mobile phase ratio (± 3 %)	40:60	98.04	98.3	0.198	0.2014
	50:50:00	98.34			
	60:40:00	98.52			
Limit					NMT 2%

System suitability test

As system suitability test was an integral part of chromatographic methods development and was used to verify that the system is adequate for the analysis to be performed, the system suitability parameters for Nefopam were evaluated.

The suitability of the chromatographic system was demonstrated by comparing the obtained parameter values, with the acceptance criteria of the ICH guidelines, such as Area, tailing factor, theoretical plates and retention time Table 8 [7].

Table8: *System suitability studies.*

Sr. No.	Area Reproducibility	Retention Time	Tailing Factor	Theoretical Plates
1	18152	5.01	1.37	9574
2	18136	5.01	1.38	9803
3	18121	5.01	1.39	9855
4	18118	5.01	1.37	9844
5	18125	5.02	1.38	9867
6	18132	5.02	1.38	9848
Mean	18131	5.01	1.378	9799
SD	12.5	0.0045	0.006872	-
% RSD	0.069	0.08983	0.498562	-
Limit	NMT 2.0 %	NMT 1 %	NMT 2 %	NLT 2000

Assay: Six sample preparations of Nefopamof 50µg/ml were prepared and injected into the chromatographic system. The Mean, Standard deviation and % RSD of Assay percentage of Nefopamin sample solution was calculated by Table 9.

Table9: *Assay of Nefopam sample.*

Sr. No.	Weight of standard (mg)	Sample Weight (equivalent to 10mg)	Area of standard at 238 nm	Area of sample at 238nm	% Assay
1	10.1	33.2	17783	17314	99.12
2		33.5		17295	99.64
3		33.1		17285	99.35

4		33.6		17285	99.35
5		33.5		17280	99.38
6		33.8		17269	99.4
Claim: 30 mg/tab Mean					99.37
Average wt. 0.1675gm				± SD	0.1672
Limit: NMT 2.0%				% RSD	0.1682
*Average of 6 determinations; SD is standard deviation; %RSD is relative standard deviation.					

Results and Discussion

Novel and simple RP HPLC method have been developed for the determination of Nefopam in Tablet dosage forms. The chromatographic conditions were optimized by taking into consideration the chemical structures of Nefopam, choice of the column with respect to chemistry of packing material, dimension of column, the composition, flow rate of mobile phase, wavelength of detection and injection volume.

The optimized chromatographic condition was found satisfactory to yield well retained, sharp and symmetrical peak at 5.17 min. The results of linearity studied over the concentration range 50-150µg/mL showed the linear detector response with correlation coefficient of 0.999 and the regression equation ofy = 0.0056×-0.7744.

Good recovery of the spiked drug was obtained by standard drug addition method at each added concentration, indicating that the method was accurate. Percent Recovery was observed to be 100.45 % representing the accuracy of the method.

Replicate estimations (n=6) of Nefopam in standard solution and tablet formulation by proposed method have yielded % RSD of 0.1938% and 0.0810%respectively indicating substantially high precision of system and method(repeatability) [8].

The intermediate precision study was ascertained on the basis of intra-day and inter-day data obtained by analysing Nefopam in Tablet dosage forms by proposed method and it is found to be very much reproducible with minimum %RSD.

The method was sufficiently robust for normally expected variations in chromatographic conditions such as wavelength, temperature and flow rate.

For specificity study reveals that the peak obtained in the standard and sample chromatogram at working concentration are only because of the drug. In blank and excipient solution, there is no peak at the retention time of Nefopam which proved that there was no interference of the blank and excipient peaks. Thus, the proposed RP-HPLC method is capable of estimating specifically the drug components in the presence of blank and other excipient peaks.

The number of Average theoretical plates was 9799 and tailing factor was 1.378 for Nefopam, which indicates efficient performance of the column. The system suitability tests are shown in Table 8.

Conclusion

The developed HPLC method is simple, specific, accurate and precise for Nefopam in tablet dosage form. It was successfully validated in terms of linearity, accuracy, precision, specificity and robustness in accordance with ICH Q2 (R1) guidelines. Also the developed method is better than earlier published articles with respect to superior System Suitability Parameter such as Theoretical plates, tailing factor. Thus the described method is suitable for routine analysis and quality control of Nefopam in Tablet dosage forms.

References

1. Aymard G, Warot D, Demolis P, et al. Comparative pharmacokinetics and pharmacodynamics of intravenous and oral nefopam in healthy volunteers. J Pharmacol Toxicol. 2003;92:279-286.
2. Ahmad M, Yaqoob M, Murtaza G. Study of pharmacokinetics and comparative bioavailability of nefopam 30 mg tablets in twelve fasting healthy pakistani male young subjects: single-dose, randomized, two-period,two-treatment and two-way cross-over design. Med Princ Pract. 2012;21:271-276.
3. Seetohul LN, Paoli GD, Drummond G, et al. Nefopam hydrochloride: A fatal overdose. J Anal Toxicol. 2015;39:486-489.
4. Huart ED, Vigneron J, Clarot I, et al. Physicochemical stability of nefopam and nefopam/ droperidol solutions in polypropylene syringes for intensive care units. Eur J Hosp Pharm 2020;27:e69-e73.
5. Shama SA, Amin AS. Spectrophotometric microdetermination of nefopam, mebevrine and phenylpropanolamine hydrochloride in pharmaceutical formulations using alizarins. Spectrochimica Acta Part A Mol Biomol Spect. 2004;60:1769-1774.
6. Fatema K, Rahman MDZ, Biswas SK, et al. Development of UV spectroscopic method for nefopam and escitalopram as inn drugs in tablet dosage form. Stamford J Pharma Sci. 2010;3(1):4-10.
7. Tracqui A, Berthelon L, Ludes B. Fatal overdosage with nefopam (acupan). J Anal Toxicol 2002;26:239-243.
8. Xiao C. A new general method for the optimization of hplc ternary or pseudo-quaternary mobile phases and the separation of two new metabolites of nefopam from greyhound urine. Oregon State University. 1991.

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