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ESTIMATION OF NEFOPAM HYDROCHLORIDE IN BULK AND PARENTERAL DOSAGE FORM BY ZERO ORDER AND AREA UNDER THE CURVE UV SPECTROPHOTOMETRIC METHODS

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ABSTRACT

Nefopam Hydrochloride is a centrally acting non-opioid analgesic drug of the benzoxazocine chemical class. It is widely used for the relief of moderate to severe pain as an alternative to opioid analgesic drugs. The aim of the present research was broadly focused on the estimation of Nefopam Hydrochloride in bulk and parenteral dosage form by using two UV Spectrophotometric methods namely, Zero order UV-Spectrophotometry (Method-1) and Area under the curve UV-Spectrophotometry (Method-2). The zero order UV-Spectrophotometric method was based on the measurement of drug absorbance at a wavelength of 266.5 nm, which was its wavelength of maximum absorbance. The area under the curve method was based on the calculation of area occupied by the UV-absorbance curve between 260-270nm. The solvent employed for both methods was distilled water. In the estimation of Nefopam Hydrochloride, both the methods showed linearity in the range of 60-300 µg/mL. The correlation coefficient was > 0.999. The precision for both the methods was <2% RSD. The accuracy was performed by using percentage recovery studies of the standard drug spiked at 75, 100 and 125% of the test concentration and the values obtained were within the limits. The developed methods were applied for the assay of the drugs in their respective dosage forms. The assay of parenteral dosage form was found to be within limits. All the results were satisfactory; the developed methods can be routinely used for the analysis of the drugs in both bulk and dosage forms.

INTRODUCTION:

In modern era, the health needs of the people were in a rise globally from time to time. The pharmaceutical industries have greater responsibilities on par with health care systems in meeting the health demands of the people. The manufacture and supply of quality pharmaceuticals is the most Important among them all. Analytical chemistry plays a vital role in the assessment of quality of the manufactured drugs and their dosage forms in the pharmaceutical sector. Thus, there is a need to develop new analytical methods, which are suitable for

the analysis of overall quality of the drugs and dosage forms. Nefopam[1,2] is a centrally-acting non-opioid analgesic drug of the benzoxazocine chemical class. It is widely used for the relief of moderate to severe pain as an alternative to opioid analgesic drugs. Nefopam has additional action in the prevention of shivering, which may be a side effect of other drugs used in surgery. Nefopam inhibits the re-uptake of various catecholamines (including noradrenaline, serotonin and dopamine). It is possible that the mechanism of action of nefopam is at least in part by altering the levels of these neuromodulators in the brain and at the spinal level. Nefopam has been shown to have sympathomimetic and anticholinergic actions. The chemical structure of Nefopam was shown in figure 1. Extensive survey of literature[3-15] on Nefopam revealed that the reported methods were mostly aimed for the clinical studies, pharmacokinetic evaluation, formulation and dissolution studies of the drug and only few analytical methods based on HPLC were reported for the quantification of Nefopam. Thus, there is a need to develop simple and economical UV methods for the quantification of Nefopam in bulk and dosage forms.

The aim of the present research was broadly focused on the estimation of Nefopam Hydrochloride in bulk and parenteral dosage form by using two UV spectrophotometric methods namely, Zero order UV-spectrophotometry (Method-1) and Area under the curve UV-spectrophotometry (Method-2).

MATERIALS AND METHODS

Instruments used:

Analytical Balance (Shimadzu, AY-220), Shimadzu UV-Visible Spectrophotometer (UV- 1800) with UV-probe data handling system, Ultra sonicator (PCI Analytics Ltd.-6.5L) and pH meter (Elico) were used in present study.

Materials used:

The working standards and parenteral dosage form of Nefopam Hydrochloride were procured from Aurobindo Pharma Ltd. Hydrochloric acid-AR grade and Sodium Hydroxide-AR grade were procured from E. Merck (India) Ltd., Mumbai. Double distilled water was obtained from in-house distillation unit.

Methods:

Zero order UV-spectrophotometric method development:

Different Solvents like Water, Methanol, 0.1N Hydrochloric acid and 0.1N Sodium hydroxide were employed for recording of the UV spectrum and for the optimization of the method.

Preparation of Stock solution

50 mg of standard Nefopam was weighed and dissolved in required amount of water in a 50 ml volumetric flask. The flask was shaken and volume was made up to the mark with water to give a solution containing 1000µg/mL (stock solution-1). Stock solution-2 was prepared by taking 1ml solution from stock solution-1 in to the 10ml volumetric flask and made up to the mark with water to produce 100µg/mL.

Calibration curve for Nefopam

From trial and error method, the concentration range from 60-300µg/ml was found to give better linearity. So, aliquots of 0.6 to 3 ml of standard stock solution-2 were transferred to a series of 10ml volumetric flasks and the final volume in each flask was made with water to obtain the concentration range from 60 to 300µg/ml. Calibration curve for Nefopam was obtained by measuring the absorbance at 266.5nm. Statistical parameters like the slope, intercept and co-efficient of correlation were determined.

Area under the Curve (AUC)-UV Spectrophotometric Method:

The principle for Area under curve method is “the area under two points on the spectra is directly proportional to the concentration of the compound of Interest”. AUC is particularly suitable for the compounds where there is no sharp peak or broad spectra were obtained, AUC method involves the calculation of integrated value of absorbance with respect to the wavelength

between two selected wavelengths ƛ1 and ƛ2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. The wavelength range was selected based on repeated observations to get the linearity between area under curve and concentration. Validation of the developed spectrophotometric methods

i. Linearity

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

ii. Range

Range is the difference between upper concentration and lower concentration. The results obtained are within the range.

iii. Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements from multiple sampling of the same homogeneous sample under prescribed conditions. The concentration of 120µg/ml, 240µg/ml, and 360 µg/ml were selected for precision. They are prepared by taking 1.2ml, 2.4ml, 3.6ml from stock solution-1 respectively into the 10ml volumetric flask and made up to the mark with water and was analyzed by using UV spectrophotometer.

iv. Accuracy

To determine the accuracy of the proposed method different levels of drug concentrations were prepared from independent stock solutions and analyzed. To provide an additional support to the accuracy of the developed assay method, a standard addition method was employed, which involved the addition of different concentrations of pure drug to a known pre-analyzed dilution of the pure drug and the total concentration was determined using the proposed method. The % recovery levels of the added sample drug were, calculated. The recovery studies were performed at different levels like 75%, 100% and 125% of the target concentration (120 mcg/ml). The amount of standard drug recovered from the spiked test samples was calculated from the absorbance values.

v. Assay Procedure

1ml of parenteral dosage form of Nefopam was taken which consists of 10mg/ml (10000µg/ml) concentration; this solution was diluted to 10ml to give the concentration of 1000µg/ml (stock solution-1). Stock solution-2 was prepared by taking 1.2ml solution from stock solution-1 in to the 10ml volumetric flask and made up to the mark with water to produce 120 µg/ml.

vi. Assay Calculation

The quantity of Nefopam was calculated from calibration curve using absorbance value of test formulation.

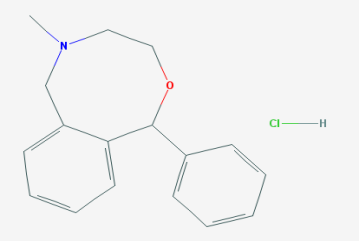

Figure 1: Chemical structure of Nefopam Hydrochloride

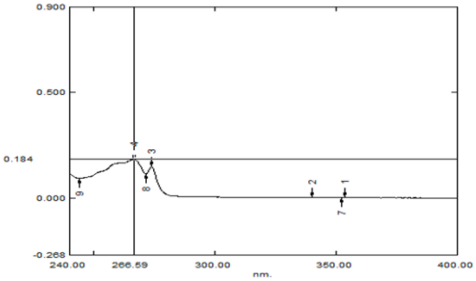

Figure 2: Maximum absorption wavelength

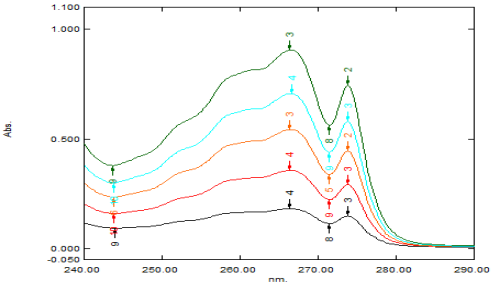

Figure 3: Overlay spectra showing linearity of Nefopam Hydrochloride

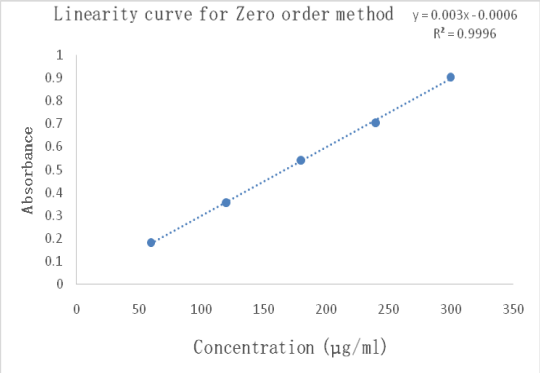

Figure 4: Linearity graph of Nefopam Hydrochloride UV-Spectrophotometry

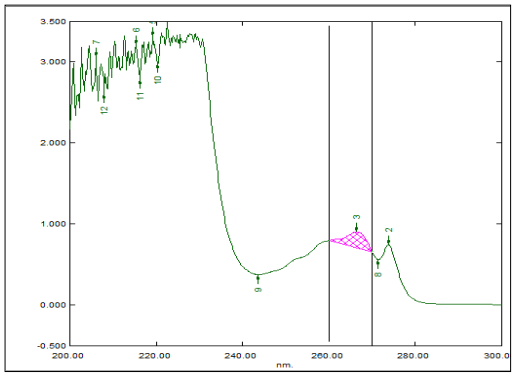

Figure 5: Selection of wavelengths for Area under the curve method

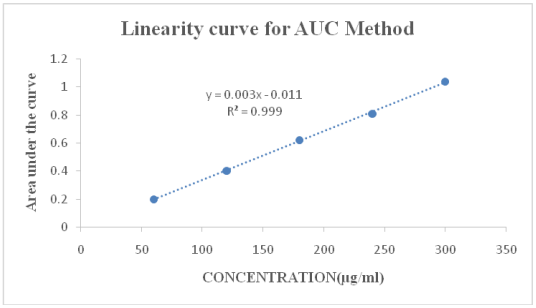

Figure 6: Linearity graph of Nefopam Hydrochloride-AUC Method

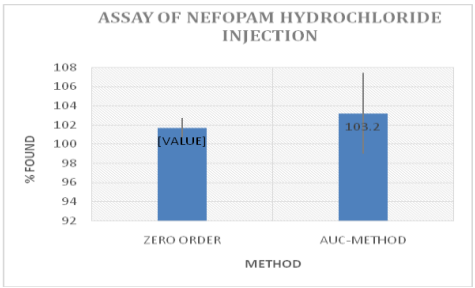

Figure 7: Comparative Assay results of two methods

RESULTS AND DISCUSSION:

Nefopam in distilled water gave a single distinct peak with good absorbance in the recorded UV spectrum, so it was employed as the solvent. The wavelength of maximum absorbance (λmax) for the bulk drug was found to be 266.5nm. The UV absorbance spectrum was shown in figure 2.

Validation of Zero order UV-Spectrophotometric Method:

i. Linearity and range:

Nefopam Hydrochloride showed good linearity in the range of 60-300µg/ml. The correlation coefficient was found to be 0.9996. The linearity data was shown in table 1 and figure 2,3&4.

ii. Precision (Repeatability):

Both intra-day and inter-day precision was within the acceptable limit with a % RSD less than 2%. So, the developed method was more precise and repeatable.

iii. Accuracy:

The recovery studies with standard addition method at 75%, 100% and 125% levels of the test concentration showed good results with a mean recovery of 100.96 %. The developed method was accurate. The results were shown in table 2.

iv. Limit of Detection and Quantification (LOD & LOQ):

The LOD was found to be 16.5 µg/ml and LOQ was 50 µg/ml.

v. Assay:

The assay of Nefopam Hydrochloride injection was calculated by using calibration curve method and was found to be 101.68 %. The consolidated results of the validation parameters of the developed method were shown in table 3.

Validation of Area under the Curve UV-Spectrophotometric Method: For the selection of analytical wavelength, 60µg/ml solution of was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200 nm to 400 nm. From the spectra of drug, area under the curve in the range of 260-270nm was selected for the analysis as shown in the figure 5. The calibration curve was prepared in the concentration range of 60-300µg/ml at their respective AUC range. By using the calibration curve, the concentration of the sample solution can be determined.

i. Linearity and range: Nefopam Hydrochloride showed good linearity in the range of 60-300µg/ml. The correlation coefficient was found to be 0.9994. The linearity data was shown in table 4 and figure 6.

ii. Precision (Repeatability): Both intra-day and inter-day precision was within the acceptable limit with a % RSD less than 2%. So, the developed method was more precise and repeatable.

iii. Accuracy: The recovery studies with standard addition method at 75%, 100% and 125% levels of the test concentration showed good results with a mean recovery of 95.76 %. The developed method was accurate. The results were shown in table 5.

iv. Assay: The assay of Nefopam Hydrochloride injection was calculated by using calibration curve method and was found to be 103.2 %. The consolidated results of the validation parameters of the developed method were shown in table 6.

CONCLUSION:

The proposed two methods based on UV spectrophotometric principles were found to be simple, economical, rapid, precise and accurate. The developed methods can be routinely used for the analysis of the drugs in both bulk and dosage forms.

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Table 1: Linearity of Nefopam Hydrochloride UV-Spectrophotometry

S.no	Concentration(µg/mL)	Absorbance at 266.5nm
1	60	0.182
2	120	0.356
3	180	0.542
4	240	0.710
5	300	0.904
Equation for regression line: y = 0.003x - 0.0006		Correlation coefficient (R^2) =0.9996

Table 2: % Recovery for Zero order UV-Spectrophotometry

Recovery range	Test Concentration (µg/ml)	Amount of standard concentration spiked (µg/ml)	Amount of sample concentration found (µg/ml)	% Recovery
75%	120	90	210.5	101.5%
			211.5	
			212.1	
100%		120	240.1	100.1%
			240.8	
			239.5	
125%		150	271.1	101.3%
			272.1	
			272.8	

Table 3: Validation parameters of developed method for Nefopam Hydrochloride UV-Spectrophotometry

S.no.	Validation Parameters	Results for Nefopam Hydrochloride
1.	Linearity	60 -300µg/ml
2.	Correlation coefficient	$R^2 = 0.9996$
3.	Regression Equation	y = 0.003x - 0.0006
4.	Slope	0.003
5.	Intercept	0.0002
6.	Precision	< 2%
7.	Intra-day precision (n = 3)	0.427 % RSD (120µg/ml)
		1.284 % RSD (180µg/ml)
		1.534 % RSD (240µg/ml)
8.	Inter-day precision (n = 3)	0.427 % RSD (1^{st} day)
		0.866 % RSD (2^{nd} day)
		0.753 % RSD (3^{rd} day)
9.	LOD	16.5 µg/ml
10.	LOQ	50 µg/ml
11.	Accuracy (Mean % Recovery)	100.96%
12.	Assay	101.68%

Table 4: Linearity of Nefopam Hydrochloride-AUC Method

S.no	Concentration (µg/mL)	AUC Between 260-270nm
1.	60	0.199
2.	120	0.402
3.	180	0.619
4.	240	0.809
5.	300	1.036
Equation for regression line: y = 0.0035x - 0.0113		Correlation coefficient (R^2) =0.9994

Table 5: % Recovery-AUC Method

Recovery range	Test Concentration (µg/ml)	Amount of standard concentration spiked (µg/ml)	Amount of sample concentration found (µg/ml)	% Recovery
75%	120	90	222.69	95.5%
			220.51	
			223.82	
100%		120	248.47	96%
			250.25	
			251.62	
125%		150	272.39	95.8%
			275.23	
			274.35	

Table 6: Validation parameters of developed method for Nefopam Hydrochloride-AUC Method

S.no	Validation Parameters	Results for Nefopam Hydrochloride
1.	Linearity	60-300µg/ml
2.	Correlation coefficient	$R^2 = 0.9994$
3.	Regression Equation	y = 0.0035x - 0.0113
4.	Slope	0.0035
5.	Intercept	-0.0113
6.	Precision	< 2%
7.	Intra-day precision (n = 3)	1.406 % RSD (120µg/ml)
		1.648 % RSD (180µg/ml)
		1.077 % RSD (240µg/ml)
8.	Inter-day precision (n = 3)	1.406 % RSD (1st day)
		1.381 % RSD (2nd day)
		0.912 % RSD (3rd day)
9.	Accuracy (Mean % Recovery)	95.76%
10.	Assay	103.2%

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