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## Nepafenac Estimation in Ophthalmic Formulations by Developing and Validating UV Spectrophotometric Method

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**ABSTRACT**

For the quantitative determination of nepafenac an easy, perfect and specific ultra violet spectroscopic approach was developed and validated. Solvent used was ethanol in this approach. In the 4 -20 µg/ml concentration range Beers law was obeyed with  $r^2 = 0.999$ . Solid linear connection with slope and intercept of 0.071 and 0.034 respectively was revealed by linear regression. The process was statistically validated and the standard deviation and RSD were in the adequate range. The proposed approach yielded a percentage recovery of the medication in the range of 98 to 100.71 % suggesting no interference from the formulation additives. After validation of the method results were found to be satisfactory. 0.371µg/ml and 1.1267µg/ml were values of LOD & LOQ respectively.

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**INTRODUCTION:**

US FDA approved the NSAIDS 0.1% diclofenac, bromfenac 0.09%, ketorolac 0.6% and 0.1% & 0.3% nepafenac ophthalmic suspensions for the treatment of inflammation and pain of eyes.<sup>1, 2</sup> Nepafenac is administered after cataract surgery as well as prophylactic. It is licensed for the cure of ocular pain & inflammation after cataract surgery and is marketed as Nevanac® suspension in 2 strengths one is 3mg/ml dosage once per day and 1mg/ml dosage one drop thrice per day.<sup>3</sup> Some of the side effects of nepafenac are eyes become sticky, poor visual acuity and increase in intra ocular pressure.<sup>4</sup> Sahu et al studied the effects on ocular inflammation by using drugs like ketorolac, bromfenac and nepafenac. It was revealed that nepafenac decreases the redness of anterior chamber more effectively

than other drugs.<sup>5</sup> Also Modi et al observed that use of 0.3% once a day was more feasible than use of 0.1% thrice a day.<sup>6</sup> After topical instillation nepafenac penetrates through the cornea & is changed amfenac by ocular tissue hydrolases which being its active metabolite.<sup>7, 8</sup> Appearance of COX enzyme has been examined extensively.<sup>9, 10</sup> Due to eye inflammation changes also occur in the ocular angiogenesis permeability changes and changes in the blood ocular barrier. Inflammation of posterior region is treated by steroidal as well as by non steroidal antiinflammatory drugs<sup>11, 12</sup>. When used repeatedly usual corticosteroids e.g. fluoromethalone, prednisolone, dexamethasone or flucinolone cause adverse affects e.g. elevated intraocular pressure, formation of cataracts, risk of infection or macular edema.<sup>13</sup> Ultra violet spectroscopic method was developed for the determination of drug in ophthalmic formulations that is simple and accurate. The proposed approach was validated as per ICH criteria.<sup>14</sup>

**Experimental:**

**INSTRUMENTS:** UV Visible spectrophotometer was used for checking the absorbance of samples.

Digital balance was used for weighing.

**Chemicals Used:** Flax Laboratories Mumbai provided drug as a gift sample. Solvent used for method validation was ethanol and was purchased from Sigma Aldrich.

**Procedure:**

1. 10 mg of drug was added to ethanol 100ml in a volumetric flask to make the stock solution having ultimate strength of 100µg/ml.
2. Sonication of stock solution was done for 20 minutes in a bath sonicator to ensure the proper dissolution of drug in the solvent.
3. Dilutions were prepared in the series of 4 to 20µg/ml and absorbance was taken using ethanol as blank.

**Determination of lambda max:** To achieve a concentration of 20µg/ml, 2ml of the above prepared stock solution was placed in a test tube and the volume was increased to 10ml using ethanol. Using ethanol as a blank, a sample from this 10ml solution was scanned in the region of 200 to 400nm. The maximum wavelength of nepafenac was discovered to be 237 nm.

Table 1 Parameters for method validation

Drug	Nepafenac
Solvent	Ethanol
Instrument	Uv spectrophotometer
lambda max	237

**Validation of the Procedure:**

The approach has been validated according to the requirements provided in the ICH recommendations, which are given below.

**Accuracy:** Analytical procedure's accuracy is expressed by the agreement of closeness amid the acknowledged values as a true conventional value or an agreed value of reference & the discovered value.

**Precision:** It expresses the closeness of agreement (degree of scatter) amid 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, inter-mediate precision and reproducibility. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurement.

Table 2 Values of absorbances

Conc. µg/ml	Absorbance 1	Absorbance 2	Absorbance 3	Mean	Standard deviation
4	0.2360	0.2380	0.2390	0.2376	0.000943

**Ruggedness:** To calculate the ruggedness wavelength was altered by plus minus 2 of 230 nm and absorbance was measured at these new wavelengths.

**Robustness:** When moderate but deliberate changes are made in the parameters of an analytical method its ability to remain unaffected is defined as robustness of the method. Fluctuations in pH in a solvent solution and temperature are two examples of normal variations.

**Specificity:** When an analyte can be definitively assessed in presence of other additives present with it is known as specificity of the method. Additives present may be in the form of impurities, matrix, degradants or other substances.

**Limit of detection (LOD):** The lowest amount of an analyte in a sample that can be detected but not necessarily quantified as an exact number is the detection limit of an individual analytical method. The detection limit can be determined using the following criteria: 1. visual evaluation 2. Noise to signal 3. The response standard deviation and the slope.

**Limit of quantitation (LOQ):** The smallest quantity of an analyte in a sample that can be quantitatively determined with sufficient precision and accuracy is the limit of quantitation of an analytical method. The quantitation limit is a parameter of quantitative tests for low quantities of chemicals in sample matrices and it is used to determine contaminants and or degradation products in particular. Visual evaluation, noise to signal and RSD and slope can be used to determine the quantitation limit.

**RESULTS AND DISCUSSIONS:**

**Method:**

Suitable aliquots were taken in 10ml test tubes from the already prepared stock solution of drug (0.4 to 2 ml). To make the concentrations in the range of 4-20 µg/ml volume of test tubes was made up to 10ml by the addition of ethanol and test were shaken for few minutes. Ethanol was used as a blank. Absorbance was taken at 237 nm by using ethanol as blank. Concentration on x axis & absorbance values on y axis are taken calibration curve was plotted and a straight line was obtained in the conc. range from 4 to 20 µg/ml. Same procedure was repeated 3 times and mean of absorbances was taken & standard deviation was calculated as well. Graph was plotted between mean absorbance and concentrations & a straight line was obtained as shown in below figure:

8	0.5573	0.5472	0.5342	0.5462	0.010889
12	0.8463	0.8352	0.8262	0.8359	0.009475
16	1.1343	1.100	1.100	1.1114	0.008085
20	1.3807	1.3707	1.3987	1.3833	0.010842

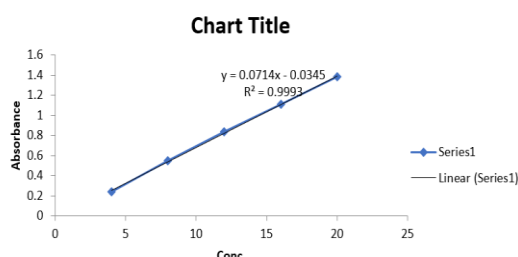


Figure 1 Calibration curve of drug in ethanol

Table 3: Parameters of the method

$\lambda_{\max}$ nm	237
Beer's law limit	4-20 (ug/ml)
R <sup>2</sup>	0.999
Intercept	-0.034
Slope	0.071
LOQ	1.126%w/v
LOD	0.3718%w/v

#### Accuracy:

Standard addition method was used for the recovery studies on 3 dissimilar levels i.e. 80,100 & 120 %. Results were satisfactory and are shown in table below:

Table 4 Recovery results

Stage	Amount of Nepafenac added (µg)	Nepafenac amount found (µg)	% Revival
80	08	7.88	98.5
100	10	9.75	97.5
120	12	11.92	99.33

#### PRECISION:

Intraday and interday assay precisions were used for the determination of reproducibility. Percent of RSD was found to be less than 2.

#### RUGGEDNESS:

Original wavelength was varied by  $\pm 20^\circ\text{C}$  i.e., 227 and 233 nm and absorbance's were taken. The results are shown in below table no.5

Table 5. Ruggedness

µg/ml	227 nm	237nm	233nm
4	0.2403	0.2371	0.2655
8	0.5494	0.5462	0.5746
12	0.8391	0.8359	0.8643
16	1.1146	1.1114	1.1398
20	1.3865	1.3833	1.4117

#### ROBUSTNESS:

To demonstrate robustness, the parameter of the UV method for the assay of Nepafenac was sequentially varied but keeping all other parameters constant. The parameters varied were temperature 40-45°C. The consequence of keeping the test solution in 40°C was given in below table No.6

Table 6: Robustness:

Parameter	Variation	% Recovered
Temperature	40°C	100.53

#### Specificity:

For specificity rationale a mixture of excipients like polymers were added to the drug using the same procedure as for stock and sample preparation and the UV spectrum was recorded for any changes. When sample was scanned from 200 to 400 there was no difference in the spectrum and method was found out to be specific.

#### Limit of detection:

Limit of detection =  $3.3 \sigma / S$

where  $\sigma$  is SD of y intercepts of regression lines, S = calibration curve slope

From calibration curves,

$\sigma = 0.0080$ ,  $S = 0.071$

$LOD = 3.3 \times 0.0080 / 0.071 = 0.3718\% w/v$

#### Limit of Quantification

Quantification limit =  $10 \sigma / S$

where  $\sigma$  is SD of y intercepts of lines of regression, s calibration curve slope. From calibration curves,

$\sigma = 0.0080$ ,  $S = 0.071$

$LOQ = 10 \times 0.0080 / 0.071 = 1.1267\% w/v$

#### Stability of Solutions

The stock solution of standard was steady for more than 24 hours when kept at room temperature while sample solution was found to be stable for not more than 6 hours at room environment but for more exact domino effect sample solution was used within 3 hours.

#### CONCLUSION:

The proposed UV spectrophotometric method for nepafenac estimation was found out to be rapid, simple & accurate as per the results. Quantitative estimation of drug in formulations having this pharmaceutical ingredient can be estimated by this method.

#### ABBREVIATIONS

UV: ultra violet

LOD: Limit of detection

LOQ: Limit of quantification

US: United States

FDA: Food and drug administration

NSAIDS: Non-steroidal anti-inflammatory drugs

COX: Cyclooxygenase

$\lambda_{\max}$ : Lamda max

R2: Regression coefficient

SD: Standard deviation

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