A Simple and Rapid Spectrophotometric Method For
The Determination of Nepafenac in Pharmaceuticals

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ABSTRACT
The simple, accurate and precise UV-Spectrophotometric method has been developed and validated for quantitative determination of Nepafenac. The method employed Phosphate buffer pH 7.4 as a solvent. The drug shows solubility in Methanol and sparingly soluble in Phosphate buffer but to avoid solvent cost and making method simpler the Phosphate buffer as solvent was selected. The proposed method obeyed the Beer’s law in the concentration range of 2-18 µg/ml. The linear regression showed good linear relationship with r² = 0.999, slope and intercept were 0.052173 and 0.090 respectively. Method was validated statistically where SD and RSD were found to be satisfactorily low. Percentage recovery of the drug for the proposed method was found in the range of 98-100.71% indicating no interference of the formulation excepients. The results of the analysis were validated with respect to accuracy, precision and recovery studies which were found to be satisfactory. LOD and LOQ for Nepafenac were found to be 0.1104 µg/ml and 0.3416 µg/ml respectively. The utility of the developed method has been demonstrated by analysis of commercial formulation containing this drug.

Key Words: Nepafenac, UV Spectrophotometry, Phosphate buffer (7.4)

INTRODUCTION
The removal of diclofenac sodium ophthalmic solution as a viable pharmacological entity in September 1999 from the US market spurred considerable interest in the general safety and effectiveness of topical ophthalmic NSAIDs for treatment of anterior segment inflammation. In late 1999 the use of topical ocular NSAIDs declined in the US as a result of incidents involving corneal melts and toxicity surrounding use of generic diclofenac. However, since the removal of diclofenac sodium ophthalmic solution from the marketplace, ophthalmic NSAIDs have regained use as viable phamacotherapeutic entities. Moreover, several new ophthalmic NSAID products have recently been introduced for commercial use in the US including the novel chemical entity nepafenac. The purpose of this report is to revisit the use of topical ophthalmic NSAIDs for the treatment of surgically induced anterior segment inflammation with a particular focus on nepafenac. Nepafenac is unique among ophthalmic NSAIDs in that it is a prodrug deaminated to amfenac, a highly effective non-selective cyclo-oxygenase inhibitor. In the case of topical ophthalmic NSAIDs, practitioners should carefully weigh the cost-benefit of implementing “highly potent” new drug products because perturbations in pharmacodynamic response due to the inherent novelty in terms of chemical designs may outweigh the demonstrated replicative pharmacologic action of all topical ophthalmic NSAIDs.

The theoretical advantage offered by nepafenac over other existing NSAIDs is in corneal penetration. However, the expected therapeutic advantage of nepafenac based on its corneal permeability profile and absorption is not fully recognizable in comparative assessment of clinical anti-inflammatory efficacy. The issue of bioactivation as it pertains to nepafenac is a key point in pharmacodynamics pertaining to the active amfenac entity. Although Nepafenac is metabolized in the mammalian iris and retina, the rate of amide hydrolysis demonstrable in human aqueous has not been fully elucidated; therefore, comparative prediction regarding peak nepafenac concentration in aqueous as an implied indicator of effectiveness in the human is at best equivocal.

Prior to use of ophthalmic non-steroidal anti-inflammatory suspensions, topical ophthalmic steroids were the mainstay treatment of post-operative, surgically induced ocular inflammation. Although considered very effective, the use of topical corticosteroids is limited by well known side effects which in some serious cases can precipitate vision loss, and limits such therapy to short, intermittent use.

Topical non-steroidal anti-inflammatory drugs (NSAIDs) are notable for a definitive lack of corticosteroid defined toxicity and have secured an important role, in some cases an undefined role, in the treatment of ocular inflammatory disease. Acute inflammation can be the result of exogenous injury either iatrogenic or due to accidents as well as of endogenous origin such as occurs in autoimmune disease. The use of the term inflammation in the context of the present report, however, will be limited to that of inflammation due to exogenous origin secondary to post-operative ophthalmic surgery related to the production of various eicosanoids. Eicosanoids are defined as prostaglandins, leukotrienes, and other compounds that are products of the action of phospholipase A2 on the cellular phospholipid membrane and are, ingeneral, derived from the production of arachidonic acid (Figure 1). It has been shown that cyclooxygenase enzymes play a key role in maintaining cellular integrity and preventing apoptosis in eukaryotes (Xiaojun et al 1995). The eicosanoid products of cyclooxygenase play a vital role in cellular homeostasis such as modulation of platelet function as well as renal regulation of salt and water.

Interestingly, NSAIDs also have been demonstrated to exert anti-inflammatory activity by mechanisms unrelated to COX inhibition through suppression of polymorphonuclear (PMN) locomotion and chemotaxis as well as by decreasing expression of inflammatory cytokines and mast cell degranulation. There is also evidence that NSAIDs exert activity as free radical scavengers, a finding that may also contribute to lessen the inflammatory response.

As each method suffers from its own limitations, so here an attempt has been made to develop a new UV spectrophotometric method for estimation of

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Nepafenac in pharmaceutical formulation with accuracy, simplicity, precision and economy.

**EXPERIMENTAL WORK**

**INSTRUMENTS**

UV- Visible double-beam spectrophotometer, Shimadzu model 1800 with spectral bandwidth of 1 nm, wavelength accuracy ± 0.3 nm and a pair of 10-mm matched quartz cells was used. All the weighing was done on electronic balance.

**2.1 CHEMICALS AND REAGENTS**

A standard gift sample of Nepafenac was provided by Enaltech Pvt Ltd, Ambernath, Mumbai. Commercially available formulations were procured from local market. Spectroscopic Grade Of KH2PO4 and NaOH Mumbai. Distilled water was used throughout the study.

**PROCEDURE:**

1. Drug solution of different concentration was prepared.
2. 100mg of drug + 100 ml of Phosphate buffer (7.4).
3. Successive dilutions were prepared as 100 ppm and 10 ppm.
4. Taking absorbance using blank prepared in same way but without drug.
5. Absorbance was taken and a further dilution was carried out according to Absorbance.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Nepafenac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent</td>
<td>Phosphate buffer (7.4)</td>
</tr>
<tr>
<td>Instrument</td>
<td>UV 1800 (Shimadzu)</td>
</tr>
<tr>
<td>Wavelength Maxima</td>
<td>234 nm</td>
</tr>
</tbody>
</table>

**DETERMINATION OF ABSORBANCE MAXIMA:**

The stock solution was further diluted with distilled water to get concentration of 10µg/ml. This solution was then scanned in the range of 200 – 400 nm where distilled water was used as a blank. The wavelength of maximum absorbance of Nepafenac was found at 234 nm.

**3. METHOD VALIDATION**

There are several parameters that are considered in the method validation process. The parameters outlined in the International Conference of Harmonization (ICH) guidelines are explained below.

**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 to ascertain the accuracy of the proposed method.

**3.2 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. 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 measurements.

**RUGGEDNESS:**

To calculate the Ruggedness, The parameters of UV method were varied by ±2 of original wavelength i.e. 234 nm. The wavelengths were change and absorbance were taken to find out any large variation in absorbance due to change in wavelength parameter.

**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. Examples of typical variations are:

- Influence of variations of pH in a solvent system
- Temperature

**SPECIFICITY:**

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

**LIMIT OF DETECTION (LOD)**

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The limit of detection can be found based on:

1. Visual Evaluation
2. Signal-to-Noise
3. The Standard Deviation of the Response and the Slope

**LIMIT OF QUANTITATION (LOQ)**

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products. The limit of quantitation may be determined by:

1. Visual Evaluation
2. Signal-to-Noise
3. The Standard Deviation of the Response and the Slope

**RESULT AND DISCUSSION**

**METHOD**

Suitable aliquots of the stock solution of Nepafenac (0.5 – 5 ml) were taken in 10 ml volumetric flasks. Flasks were shaken for few minutes and volume was then made up to the mark with Phosphate buffer (7.4) to prepare a series of standard solutions containing 2-18 µg/ml in the concentration range. Absorbance was measured at 234 nm against blank. Blank was prepared by taking Phosphate buffer (7.4). Then calibration curve was plotted for Nepafenac in the concentration range of 2-18 µg/ml at 234 nm as shown in Fig. 1 and Table 1.

![calibration curve](image)

**Fig. 1: Calibration curve of Nepafenac at 234nm**
**ACCURACY**

Recovery studies were carried out by standard addition method at three different levels (80%, 100% & 120%). The results of recovery studies, expressed as percent recovery, were satisfactory and are presented in Table 2.

**Table 2: Result of Recovery studies**

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Recovery (%)</th>
<th>Fixed amount added (µg)</th>
<th>Amount Estimated (µg)</th>
<th>Recovery (%)</th>
<th>S.D.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80</td>
<td>5</td>
<td>12</td>
<td>98.5833</td>
<td>0.202262</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>5</td>
<td>14</td>
<td>99.57917</td>
<td>0.133030</td>
</tr>
<tr>
<td>3</td>
<td>120</td>
<td>5</td>
<td>16</td>
<td>100.7167</td>
<td>0.053597</td>
</tr>
</tbody>
</table>

*Mean of six determinations*

**PRECISION**

The reproducibility of the proposed methods was determined by analyzing at different time intervals on the same day (Intra-day assay precision) and on three different days (Inter-day assay precision). The relative standard deviation of their percentage contents was calculated. Percent of RSD for intra-day and inter-day assay precision was found to be less than 2.

**RUGGEDNESS:**

The variations in wavelength were carried out by ± 2°C i.e. 231 and 237 nm and absorbance were taken. Results are given in Table No:-3.

**Table 3. Ruggedness**

<table>
<thead>
<tr>
<th>µg/ml</th>
<th>231 nm</th>
<th>234nm</th>
<th>237nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0.4014</td>
<td>0.3982</td>
<td>0.3998</td>
</tr>
<tr>
<td>12</td>
<td>0.724</td>
<td>0.7238</td>
<td>0.6669</td>
</tr>
<tr>
<td>14</td>
<td>0.8371</td>
<td>0.8193</td>
<td>0.772</td>
</tr>
<tr>
<td>16</td>
<td>0.9012</td>
<td>0.9219</td>
<td>0.8342</td>
</tr>
</tbody>
</table>

**ROBUSTNESS:**

To demonstrate robustness, the parameter of the UV method for the assay of Nepafenac were sequentially varied but keeping all other parameters constant. The parameters varied were: Temperature 40-45°C. The result of keeping sample in 40°C was given in Table No:-4.

**SPECIFICITY**

To demonstrate specificity, a solution containing a mixture of the excipients was prepared using the sample preparation procedure and the UV spectrum of this solution was recorded in the range of 200–400 nm for any interferences. The absorption spectra was taken there was no difference in spectra and the method was found to be specific.

**LIMIT OF DETECTION**

LOD = 3.3σ/S

Where,

\[ \sigma = \text{the standard deviation of y-intercepts of regression lines} \]
\[ S = \text{the slope of the calibration curve} \]

From calibration curves,

\[ \sigma = 0.001343, S = 0.0405 \]
\[ \text{LOD} = 3.3 \times 0.001343 / 0.0405 = 0.110466\% \text{ w/v} \]

**LIMIT OF QUANTIFICATION**

LOQ = 10σ/S

Where,

\[ \sigma = \text{the standard deviation of y-intercepts of regression lines}, S = \text{the slope of the calibration curve} \]

From calibration curves,

\[ \sigma = 0.001343, S = 0.0405, \text{LOQ} = 10 \times 0.001343/0.0405 = 0.3416 \% \text{w/v} \]

**STABILITY OF SOLUTION**

Standard stock solution was found to stable for more than 24 hours at RT while sample solution was found to be stable for not more than 6 hours at RT but for accuracy sample solution has to be used within 3 hours.

**APPLICATION:**

The 10 ppm solution of “Nepaflam” [Ajanta Pharmaceuticals, Ltd, Andheri(W)] suspension in Phosphate Buffer (7.4) was prepared and absorbance was carried out to check the utilization of this method. There was no difference in reading and the method was found to be specific.

**ACKNOWLEDGEMENTS**

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**CONCLUSION**

Based on the results obtained, it can be concluded that the proposed UV spectrophotometric method for determination of Nepafenac is rapid, economical, accurate and precise. The utility of the developed method has been demonstrated by analysis of formulation. Hence, the proposed method can be used for quantitative determination of pharmaceutical formulation containing this ingredient.

**REFERENCES**

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