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SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF NEPAFENAC IN OPTHALMIC FORMULATION

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ABSTRACT

The simple, accurate and precise UV-Spectrophotometric method has been developed and validated for quantitative determination of Nepafenac in opthalmic preparations. Two methods are proposed which employ Artificial Tear Fluid as a solvent. The drug is sparingly soluble in Artificial Tear Fluid but to avoid solvent cost and making method simpler the Artificial Tear Fluid as solvent was selected and the solubility of Nepafenac was enhanced by using SBE-beta-Cyclodextrin (Method B). Another reason for selecting ATF as a solvent was to determine the suitability of formulating Nepafenac in opthalmic preparation. The proposed methods obeyed the Beer's law in the concentration range of 2-14 µg/ml. The linear regression showed good linear relationship with r^2 =0.9990 and 0.9987, slope=0.07071 and 0.06485. Intercept were 0.16666 and -0.1237. Method was validated statistically where SD and RSD were found to be satisfactorily low. The results of the analysis were validated with respect to accuracy, precision and recovery studies which were found to be satisfactory. LOD for Nepafenac was found to be 1.524327003 and 0.504911475 µg/ml and LOQ was found to be 4.619172735 and 1.530034774 µg/ml respectively.

KEYWORDS: Nepafenac, UV Spectrophotometry, Artificial Tear Fluid.

INTRODUCTION

Nonsteroidal Anti-inflammatory Drugs (NSAIDs) have been used to treat various diseases for over 100 years. These drugs show anti-inflammatory, anti-allergic, analgesic and antipyretic activity and widely used to treat chronic inflammatory states, such as arthritis, psoriases and asthma. Since the introduction of topical Indomethacin for use in ophthalmic disease, several

generations of NSAID have been brought to market. One of the more recent products of the NSAID class approved for topical opthalmic use is Nepafenac, a prodrug of Amfenac for the treatment of post-operative inflammation after cataract surgery. 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. Still there is no USP and BP method is available for the analysis of drugs for its purity as well as for content assay in its dosage form. 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 Nepafenac in opthalmic preparation 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.

CHEMICALS AND REAGENTS

A standard gift sample of Nepafenac was provided by Ajanta pharma. Spectroscopic grade of NaCl, $NaHCO_3$ and $CaCl_2$ was obtained from Merck with AR grade, distilled water was used throughout the study.

PROCEDURE

1. Preparation of ATF (Artificial Tear Fluid) solution as per the formula-

Sr no.	Ingredients	Qty taken
1.	NaCl	3.35 g
2.	$NaHCO_3$	1 g
3.	$CaCl_2$	0.04 g
4.	Water	Upto 500 ml

2. 50 mg of drug + 50 ml of Artificial Tear Fluid.(1 mg/ml) and 50 mg of drug+1.297 g of SBE-β-CD + 50 ml of Artificial Tear Fluid in two separate volumetric flasks followed by sonication.

3. Successive dilutions were prepared as 100µg/ml and 10µg/ml of both the solutions.

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.

Drugs	Nepafenac
Solvent	Artificial Tear Fluid
Instrument	UV 1800 (Shimadzu)
Wavelength Maxima	238 nm

DETERMINATION OF ABSORBANCE MAXIMA

The stock solution was further diluted with ATF to get concentration of 6µg/ml. This solution was then scanned in the range of 200 – 400 nm where ATF was used as a blank. The wavelength of maximum absorbance of Nepafenac was found at 238 nm.

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.

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, 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 measurements.

RUGGEDNESS

To calculate the Ruggedness, The parameters of UV method were varied by ±2 of original wavelength i.e. 238nm.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 and 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 A

For Nepafenac in ATF:

Suitable aliquots of the stock solution (20µg/ml) of Nepafenac (1 – 6 ml) were taken in 10 ml volumetric flasks. Flasks were shaken for few minutes and volume was then made up to the mark with ATF to prepare a series of standard solutions containing 2-14 µg/ml in the concentration range. Absorbance was measured at 238 nm against blank. Blank was prepared by taking ATF. Then calibration curve was plotted for Nepafenac (without CD) in the concentration range of 2-14 µg/ml at 238 nm as shown in Fig. 1 and Table 1.

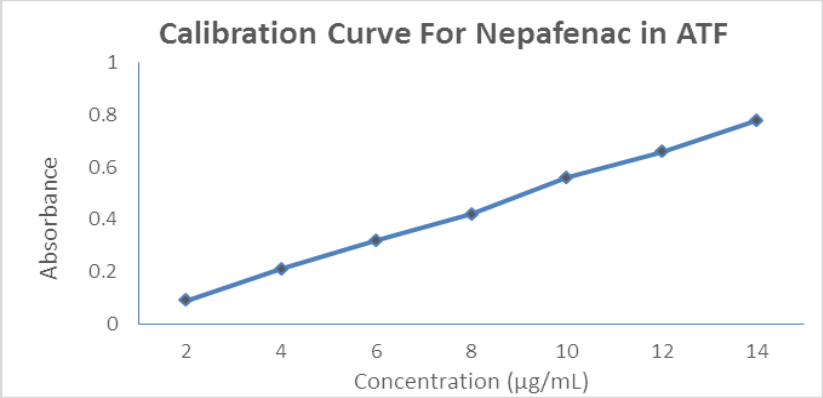

Fig. 1: Calibration Curve for Nepafenac in ATF

METHOD B

For Nepafenac with SBE-β-CD in ATF

Same procedure was employed only except the first step i.e. preparation of stock solution in which drug addition in ATF was followed by the addition of 1.297g of SBE-β-CD. Successive dilutions were made in the same manner as above and calibration curve was plotted as shown in Fig. 2

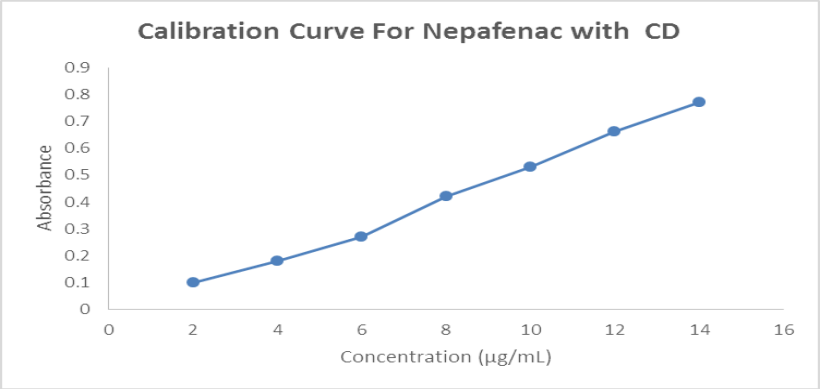

Fig. 2: Calibration Curve for Nepafenac in ATF

Table 1: Optical characteristics and Other Parameters

Parameters	UV Spectrophotometric Method	
	METHOD A	METHOD B
λ max (nm)	238 nm	238 nm
Beer's Law limit (ug/ml)	2-14 µg/ml	2-14 µg/ml
Correlation Coefficient r2	0.999500573	0.99721761
Slope (m)	0.057321429	0.057678571
Intercept (c)	-0.024285714	-0.042857143
LOD ug/ml	1.524327003	0.504911475
LOQ ug/ml	4.619172735	1.530034774

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

Sr no.	Level of recovery %	Fixed amount added(µg)	Amount added (µg)	Amount estimated (µg)	Recovery %	(±)S.D	%RSD
1	80	2	2.001	1.97	98.45077461	0.064780328	0.5
2	100	4	4.001	3.99	99.72506873	0.064780328	0.57
3	120	6	6.002	5.98	99.63345551	0.836281686	0.61

PRECISION

The reproducibility of the proposed methods was determined by analyzing at different time intervals on 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 7.

RUGGEDNESS

The variations in wavelength were carried out by ± 2 nm i.e. 236 and 240 nm and absorbance were taken. Results are given in Table No:-3

Table3. Ruggedness

µg/ml	236nm	238nm	240nm
4	0.17	0.18	0.12
6	0.28	0.27	0.24
8	0.41	0.42	0.40
10	0.17	0.18	0.13

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^{0}C.The result of keeping sample in 40^{0}C was given in Table No:-4

Table: 4. Robustness

Parameter	Variation	% recovered
Temperature	40^{0}C	97%

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,

σ = the standard deviation of y-intercepts of regression lines S = the slope of the calibration curve From 1st calibration curve, σ = 0.026477758, S = 0.057321429

LOD = 3.3 × 0.026477758/0.057321429= 1.524327003% w/v

From 2nd calibration curve, σ =0.008825022, S =0.057678571

LOD = 3.3 ×0.008825022/0.057678571= 0.504911475% w/v

LIMIT OF QUANTIFICATION

LOQ = 10σ/S Where,

σ = the standard deviation of y-intercepts of regression lines, S = the slope of the calibration curve From 1st calibration curve, σ =0.026477758, S = 0.057321429

LOQ = 10 × 0.026477758/0.057321429= 4.619172735%w/v

From 2nd calibration curve, σ =0.008825022, S = 0.057678571

LOQ = 10 ×0.008825022/0.064857143 =1.530034774 %w/v

CONCLUSION

Based on the results obtained, it can be concluded that the proposed UV spectrophotometric methods for determination of Nepafenac are rapid, economical, accurate and precise. Hence, the proposed methods can be used for quantitative determination of pharmaceutical formulation containing this ingredient.

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