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TWO VISIBLE SPECTROPHOTOMETRIC METHODS FOR DETERMINATION OF NEPAFENAC IN OPHTHALMIC SUSPENSION

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ABSTRACT

Two simple and sensitive visible spectrophotometric methods have been developed for the estimation of nepafenac in pure and pharmaceutical dosage forms. These methods are based on the diazotization coupling reaction between NED and nepafenac resulting in the formation of pink colored chromogen (λ_{max} 530 nm) and the redox reaction between FC reagent and nepafenac resulting in the formation of bluish green colored chromogen (λ_{max} 740 nm). The absorbance was measured against the corresponding reagent blanks. These methods have been statistically evaluated and found to be precise and accurate.

Key words: Nepafenac, Spectrophotometry, Ophthalmic suspension.

INTRODUCTION

Nepafenac is chemically 2-Amino-3-benzoylbenzene acetamide. This novel pro-drug rapidly penetrates ocular tissues and is converted intraocularly from nepafenac to amfenac, a potent NSAID. A number of methods such as HPLC was reported for the estimation of nepafenac in its pure form and pharmaceutical formulations. Literature survey reveals that visible spectrophotometric methods have not been reported for its quantitative determination of nepafenac in its pure form and pharmaceutical formulations. In the present investigation, two simple and sensitive visible spectrophotometric methods have been developed for the determination of nepafenac. The developed methods involve the formation of colored chromogens with NED and FC reagent. These colored chromogen showed absorption maximum at 530 nm and 740 nm, respectively. Beer's law is obeyed in the concentration

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range of 10-30 µg/mL and 10-30 µg/mL. The results of analysis for the these methods have been validated statistically and by recovery studies.

EXPERIMENTAL

Preparation of reagents

(i) NED reagent: 0.1 g of NED was dissolved in 100 mL distilled water.

(ii) Sodium nitrite solution: 0.1 g of sodium nitrite was dissolved in 100 mL of distilled water.

(iii) Ammonium sulfamate solution: 0.5 g of ammonium sulfamate was dissolved in 100 mL distilled water.

(iv) 5M HCl: 4.25 mL conc HCl was diluted with 100 mL distilled water.

(v) Standard drug solution (For method A): Accurately weighed 100 mg of nepafenac was dissolved in 100 mL distilled water from which, 1 mL was diluted to 10 mL with water.

(vi) FC reagent preparation: 1 part of FC reagent solution was mixed with 2 parts of distilled water.

(vii) Sodium carbonate solution: 10 g of sodium carbonate was dissolved in 100 mL distilled water

(viii) Standard drug solution (For Method B): Accurately weighed 100 mg of nepafenac was dissolved in 100 mL distilled water from which, 1 mL was diluted to 10 mL with water.

Assay procedures

Method A: Aliquots of working standard solution of nepafenac ranging from 1-3 mL were transferred into a series of 10 mL volumetric flasks. To these, 1 mL of 5M HCl and sodium nitrite solutions were added and shaken for 2 min. Then 1 mL each of ammonium sulfamate and NED reagent were added. Finally, it was made upto 10 mL with water. The absorbance of the pink colored chromogen was measured at 530 nm against reagent blank and the amount of nepafenac present in the sample was computed from its calibration curve.

Method B: Aliquots of working standard solution of nepafenac ranging from 1-3 mL

were transferred into a series of 10 mL volumetric flasks. To these, 1 mL of FC reagent and 2 mL sodium carbonate (10% w/v) solution were added and finally, volume was made up to 10 mL with water.

RESULTS AND DISCUSSION

The optical characteristics such as Beer's law limits, Sandell's sensitivity, molar extinction coefficient, percentage relative standard deviation and percentage range of error (0.05-0.01) were calculated for these methods and the results are summarized in Table 1. The values obtained for the determination of nepafenac in pharmaceutical formulation (ophthalmic suspension) by the proposed method is presented in Table 2. Studies reveal that the common excipients and other additives usually present in the suspension did not interfere in the proposed methods.

Table 1: Optical characteristics, precision and accuracy of the proposed method

Parameters	Method A	Method B
λ_{max} (nm)	530	740
Beer's law limit (µg/mL)	10-30	10-30
Sandell's sensitivity (µg/cm^2/0.001 abs. unit)	0.040	0.043
Molar absorptivity(litre.mole^{-1} cm^{-1})	0.0006×10^4	0.0005×10^4
Regression equation (Y*)		
Slope (b)	0.019	0.019
Intercept (a)	0.047	0.044
Correlation coefficient (r)	0.9994	0.9998
% Relative standard deviation	0.535	0.681
% Range of error		
0.05 Significance level	0.447	0.569
0.01 Significance level	0.658	0.837

Y* = a + bx, where Y is absorbance and x is concentration of nepafenac in µg/mL.

Table 2: Estimation of nepafenac in pharmaceutical formulations

Formulations (Ophthalmic suspension)	Labelled amount (mg/mL)	Amount found* by proposed method		% Recovery** by proposed method	
		Method A	Method B	Method A	Method B
Sample 1	1	98	99.2	98.3	99.13
Sample 2	1	99.2	98.6	99.62	99.23
Sample 3	1	96.4	97.7	98.26	98.8

*Average of determinations

**Recovery of amount added to the pharmaceutical formulation (Average of three determinations)

CONCLUSION

The proposed methods are applicable for the assay of drug nepafenac and have an advantage of wider range under Beer's law limits. The proposed methods are simple, selective and reproducible and can be used in routine determination of nepafenac in pure form and formulation with reasonable precision and accuracy.

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