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NOTE

Visible Spectrophotometric Determination of Apraclonidine in Dosage Form

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Two simple colorimetric methods for the estimation of apraclonidine in dosage from were developed. In method A apraclonidine undergoes reaction with *p*-dimethylaminobenzaldehyde in acidic medium to form chromogen with λ_{max} at 450 nm and obeyed Beer's law in the concentration range of 4-24 µg/mL. In the method B based on the formation of diazotized coupling reaction with sodium nitrite, hydrochloric acid and chromotropic acid. The orangish chromogen had 529 nm as λ_{max} with apparent molar absorptivity. The proposed methods gave precise and reproducible results for the estimation of apraclonidine from its dosage form.

Key Words: Spectrophotometric analysis, Apraclonidine.

Apraclonidine[1,2] is chemically *p*-aminoclonidine, used in treatment of post surgical elevated intraocular pressure. The effect of apraclonidine was proved by clinical reports[3] but analytical methods not have been reported for the formulation study. The present work describes two spectrophotometric methods for the estimation of apraclonidine in its formulation.

All spectral and absorbance measurements were done on a Jasco-Double Beam UV-Visible spectrophotometer (Model V-530) with 1 cm matched cuvettes. All reagents were freshly prepared, all chemicals used were of AR grade from SD Fine Chemicals Ltd., Mumbai, India.

Reagents like sodium nitrite (3 % w/v), sulphuric acid (1 M), sulphamic acid (5 % w/v), 4 M NaOH, chromotropic acid (0.2 % w/v), 1 N HCl, *p*-dimethylaminobenzaldehyde (0.2 % w/v) were prepared appropriately in Milli Q water.

Preparation of standard and sample solution: A standard stock solution of 1 mg/mL of apraclonidine was prepared in water. The working standards of 100 and 200 µg/mL were prepared by further dilution and used for method A and B, respectively.

For the analysis of formulation of apraclonidine not less than 10 eye drops containing 0.5 % w/v of apraclonidine were taken and transferred into 100 mL volumetric flask. Content of mixture was diluted with water and made up to mark. An approximate concentration equal to 100 and 200 µg/mL were prepared and used for the analysis.

Estimation procedure

Method A: Aliquots of apraclonidine (working standard) from 0.4-2.4 mL (4-24 µg/mL) were transferred into a series of 10 mL standard flask. To each of the flask, 4 mL of *p*-dimethylaminobenzaldehyde (0.2 % w/v) and 2 mL of 1 M H_2SO_4 were added. All solutions were heated on a water bath maintained at 40 ºC for 20 min. After cooling the solution were made up to the mark with water. The sample solution was also subjected in same way. All standard and sample were scanned and absorbance noted at 450 nm against reagent blank. Amount of apraclonidine present in sample was computed from calibration curve.

Method B: Into a series of 25 mL flasks, aliquots of working standard solution 0.2 to 1 mL (1.6-8.0 µg/mL) was pipetted out. To each of the flask, 1 mL of $NaNO_2$ and 2.5 mL of 1 M HCl were added and kept in the ice bar for 5 min. To each flask 4 mL of sulphamic acid, 2.5 mL of chromotropic acid and 1.5 mL of 4 N NaOH were added and the volume was made up with water. All standards and sample measured at 529 nm and amount of apraclonidine present in the formulation was determined using calibration curve.

To test the accuracy and reproducibility of the proposed methods, recovery experiments were carried out by adding known amounts of the drug to the pre-analyzed formulation and reanalyzing the mixture by proposed methods. The results are summarized in Table-1.

TABLE-1
ANALYSIS DATA OF APRACLONIDINE FORMULATION

Formulation	Labeled claim (% w/v)	Amount found* (% w/v)		Recovery* (%)	
		A	B	A	B
Eye drops	0.5	0.502 ± 0.21	0.493 ± 0.47	96.40 ± 0.38	95.34 ± 0.12

*Average of ± SD of determination.

Stability of promotion was carried out by measuring the absorbance values at the time intervals of 10 min and it was found to be stable for 2 h and 3 h for methods A and B, respectively. The optical characters, regression analysis, sensitivity are carried out on 6 replicate readings and show in Table-2.

TABLE-2
OPTICAL CHARACTERS AND PRECISION

Parameters	Method A	Method B
λ_{max} (nm)	450	529
Beer's law (μg/mL)	4-24	1.6-8.0
Molar absorptivity ($g \times 10^4$)	1.3561	2.6297
Sandell's sensitivity (μg/cm^2 0.001 abs)	0.0240	0.0160
Regression equation (Y*)		
Slope (b)	0.0456	0.0772
Intercept (a)	0.0302	0.0568
Correlation coefficient (r)	0.9998	0.9999
RSD (%)	0.2346	0.1923

Y* = a + bC, where Y* = absorbance at respective λ_{max} and C = concentration of apraclonidine in μg/mL.

The sensitivity of a both methods is good. The analysis result are correlated well with label claim. The reproducibility and accuracy of a methods were found to be good as the evident SD less than 2. Accuracy was studied by percentage recovery and the results indicate non interference from excipients used in the formulation. Hence the developed methods are simple, sensitive, accurate and precise and can be applied for the routine analysis of apraclonidine from bulk and dosage form.

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