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Validated UV-spectrophotometric method for the estimation of naftopidil in bulk and tablet formulation

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ABSTRACT

New, simple, sensitive, cost effective and reproducible UV-spectrophotometric method was developed and validated for the estimation of naftopidil in bulk and tablet formulations. Naftopidil was estimated at 280 nm using acetonitrile-hydrochloric acid (pH 1.2, 100 mM) (25:75, v/v) as solvent system. This method was tested and validated for various parameters as per USP requirements and ICH guidelines.

Key words: Naftopidil, analytical method validation, UV method, spectrophotometry.

INTRODUCTION

Naftopidil (Fig. 1), 4-(2-methoxyphenyl)-a-[(1-naphthalenyloxy)-methyl]-1- piperazineethanol, is alpha 1-adrenoceptor antagonist and is new drug for the bladder outlet obstruction in patients with benign prostatic hyperplasia[1]. A survey of literature has not revealed any simple UV-spectrophotometric method for the estimation of naftopidil in bulk and tablet formulations. Phosphorimetric method [2] and high performance liquid chromatography (HPLC) method and its estimation in biological samples [3,4] were reported. But, chromatographic analyses are time consuming, costly and require expertise. A simple, sensitive and accurate UV20 spectrophotometric method can be highly useful for routine analysis of bulk and tablet formulations.

The objective of the present study was to develop simple, accurate and cost effective analytical method for estimation of naftopidil in bulk and tablet formulations. The developed method was validated as per USP requirements and ICH [5]. Statistical tests at 5% level of significance were performed on validation data [6,7].

MATERIALS AND METHODS

2.1 Instruments

A double-beam LabIndia UV/Vis spectrophotometer, model UV 3092, connected to computer loaded with UV Win 5.2.0.110 software. For the measurement of pH, LabIndia PH/CON Meter was used. For weighing, Denver Instrument, SI-234 balance was used.

2.2 Materials & Reagents

Formulations containing naftopidil: Naftomax 50, labeled to contain 50 mg of naftopidil per tablets, (Sun Pharma, Mumbai) and Nafodil, labeled to contain 75 mg of naftopidil per tablet, (Intas Pharmaceuticals Ltd, Ahmedabad). Acetonitrile (HPLC Grade) was purchased from SD Fine Chemicals, Mumbai. Hydrochloric acid and common formulation excipients such as starch, lactose, microcrystalline cellulose, dicalcium phosphate, talc, polyvinyl pyrrolidine, aerosol, crosscarmellose sodium, magnesium stearate, hydroxyl propyl methyl cellulose, ethyl cellulose and iron oxide were of analytical grade.

2.3 Analytical method development

Different solvents were investigated to develop UV spectrophotometric method for the estimation of naftopidil in tablet formulations. For the selection of media the criteria employed were sensitive, simple, cost effective, steps involved in sample preparation, solubility of naftopidil in different solvent systems, stability in different solvents and applicability of method to various purposes. Absorbance of naftopidil in the selected solvent system at respective wavelength (λ max) was determined and apparent molar absorptivity was calculated according to standard formulae (Table 2).

2.4 Calibration standards

Three stock solutions of 100 µg ml-1 of naftopidil was 50 prepared by dissolving 25 mg in 250 ml of acetonitrile-hydrochloric acid (pH 1.2, 100 mM) (25:75, v/v). For the preparation of different calibration standards, aliquots of stock solutions were transferred into a series of 10 ml standard flasks and final volume made with respective medium. Five different concentrations were prepared in the range of 5-45 µg ml-1 54 of naftopidil in the acetonitrile55hydrochloric acid (pH 1.2, 100 mM) (25:75, v/v) for standard curve and estimated at 280 nm (Table 1).

2.5 Analytical method validation

2.5.1 Specificity and selectivity

Naftopidil solutions (25 µg ml^{-1}) was prepared in selected medium along with and without common pharmaceutical excipients starch, lactose, microcrystalline cellulose, dicalcium phosphate, talc, polyvinyl pyrrolidine, aerosol, crosscarmellose sodium, magnesium stearate, hydroxyl propyl methyl cellulose, ethyl cellulose and iron oxide separately. All the solutions were scanned from 400 nm to 200 nm at a speed of 1 nm sec^{-1} and checked for any change in absorbance at specific wavelength. In a separate study, drug concentration of 25 µg ml^{-1} was prepared independently from pure naftopidil stock and commercial sample stock in selected media and analyzed (N = 10). Paired t-test at 95% level of significance was performed to compare the means of absorbance (Table 2).

2.5.2 Accuracy

Accuracy was determined by taking three different levels of drug concentrations-lower, intermediate and higher concentration (LC, IC and HC) from stock solutions and analyzed (N = 9). Accuracy was measured as the percentage relative error and mean %recovery (Table 3). An additional support to accuracy of the developed assay method, standard addition method was performed. In this study, different concentrations of pure drug (10, 20 and 30 µg ml^{-1}) were added to a known concentration of formulation sample and the total concentration was determined using the proposed method (N = 9). The percent recovery of the added pure drug was estimated as, %Recovery = [(Cs-Cu)/Ca] × 100, where Cs is the total drug concentration measured after standard addition; Cu, drug concentration in the formulation; Ca, drug concentration added to formulation (Table 4).

2.5.3 Precision

Repeatability was calculated by taking different levels of concentrations, prepared from pure drug stock solution and analyzed (N = 9) (Table 3). Intermediate precision was calculated by taking the variations of inter-day and intra-day response. Respective concentrations from stock solution in triplicates were prepared three different times in a day and studied for intra day and inter-day variation (N = 27). The relative standard deviation (%R.S.D.) of the estimated concentrations from the regression equation was taken as precision (Table 5).

2.5.4 Linearity

Linearity of the proposed method was determined by taking nine separate series of solutions of naftopidil (5-45 µg ml^{-1}) in acetonitrile-hydrochloric acid (pH 1.2, 100 mM) (25:75, v/v) were prepared from the pure drug stock solution and analyzed. Least square regression analysis was performed for the obtained data. One-way ANOVA test

was performed based on the absorbance values obtained for each level of pure drug concentration during the replicate measurement of the standard solutions (Table 2).

2.5.5 Detection limit (DL) and quantitation limit (QL)
The DL and QL of naftopidil by proposed method was determined using calibration standards. DL and QL was calculated as 3.3σ/S and 10σ/S, respectively, where σ is the standard deviation of y-intercept of regression equation and S is the slope of the calibration curve [6,7]\. (Table 2).

2.5.6 Robustness
Robustness of the proposed method was performed by (a) changing pH of the media by ± 0.1 units (b) changing the concentration of acetonitrile by ± 1% volume and (c) stability of naftopidil in the selected medium at room temperature for 48 h. Three different concentrations (LC, IC and HC) were prepared in medium with different pH and % volumes. Mean percentage recovery was determined (Table 2).

2.6 Estimation from tablet formulations
Twenty tablets were weighed, pulverized and amount of the powder equivalent to 10 mg of naftopidil was taken and extracted with medium and kept in bath sonicator for 30 min. These solutions were filtered through Whatman filter paper 1 and the filtrate was suitably diluted to prepare a 10 µg ml^{-1} concentration and the samples were analyzed using proposed analytical method. The *t*-test and *F*-test was performed in comparison with standard 10 µg ml^{-1} and the values were shown in Table 5.

RESULTS AND DISCUSSION

To select suitable solvent for estimation of naftopidil, various aqueous media like acetate buffers (pH 3.6-5.8), phosphate buffers (pH 5.8-8.0) and 100 mM sodium hydroxide were investigated. Naftopidil was shown the stable UV absorption spectra (Fig. 2). The final decision of using acetonitrile-hydrochloric acid (pH 1.2, 100 mM) (25:75, v/v) as a medium was based on criteria like: sensitivity of the method, stability of drug in selected solvent system, cost, ease of preparation and suitability of the method to dissolution studies. The λmax of naftopidil was found to be 280 nm. Absorption spectra of naftopidil at 0 time and 48 h time are overlaying in medium (Fig. 3). Statistical parameters were shown in (Table 2).

3.1 Calibration curve
The linear regression equation obtained was: absorbance at 280 nm, absorbance = [0.0208 × concentration in µg ml^{-1}] + 0.0123; with a regression coefficient of 0.9997 (Table 2).

3.2 Analytical validation
3.2.1 Specificity and selectivity
The UV-spectrum of naftopidil was not changed in the presence of common pharmaceutical excipients in selected medium. Absorption spectrum of pure naftopidil sample was matching with the marketed tablet formulation in the selected medium (Fig. 4). The calculated t-values were found to be less than that of the critical *t*-value, indicating that there was no significant difference between average absorbance of solutions prepared from pure naftopidil sample and marketed tablet formulation sample (Table 2). Therefore, proposed method is selective and specific for naftopidil estimation.

3.2.2 Accuracy
Accuracy was ranged from -0.35 to 0.29 in the selected medium (Table 3). The excellent mean %recovery (close to 100%) and low standard deviation (S.D. < 1.0) represent accuracy. The reliability and validity of the proposed method was determined by recovery studies of standard addition procedure (Table 4). The mean %recoveries (S.D) for lower, intermediate and higher concentrations were found to be 100.33 (1.0), 100.12 (0.97) and 100.19 (0.29) respectively. These results have revealed that any small change in the naftopidil concentration in the solution can be accurately determined by the proposed method.

3.2.3 Linearity
The linearity range was found to be 5-45 µg ml^{-1} 141 at 280 nm in the selected medium. Lower values of statistical parameters like standard error of slope and intercept (Table 2) indicated high precision of the proposed method.

Also, the average slope and intercept values are within the 95% confidence interval. Goodness of fit of the linear regression equation was supported by lower calculated *F*-value and high regression coefficient value.

3.2.4 DL and *QL*

DL and QL were found to be 0.68 µg ml^{-1} and 2.08 µg ml^{-1} in selected medium, respectively.

3.2.5 Robustness

Variation of pH of the selected medium by ± 0.1 did not have any effect on absorbance. The mean %recovery was found to be 100.29 ± 1.33 in the selected medium (Table 2). The drug in selected media exhibited no spectrophotometric change for 48 h when kept at room temperature (Fig. 3).

3.3 Estimation of tablet formulations

The assay values of naftopidil in different tablet formulations ranged from 100.72 ± 1.14 to 101.39 ± 0.71 with relative standard deviation not more than 1.13%. Assay values of formulations were same as label claim; this indicated that the interference of excipients is insignificant in determination of naftopidil by proposed method.

Fig. 1. Structure of naftopidil

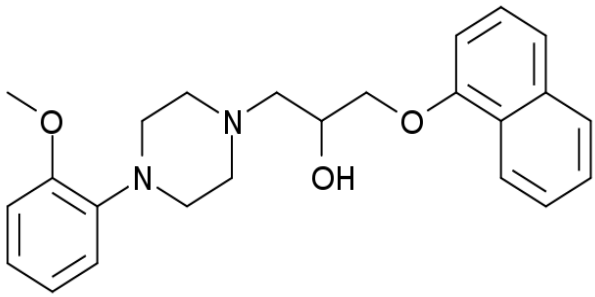

Fig. 2. Overlaid UV-spectra of naftopidil in the selected medium- the concentrations from 5 – 45 µg ml^{-1}

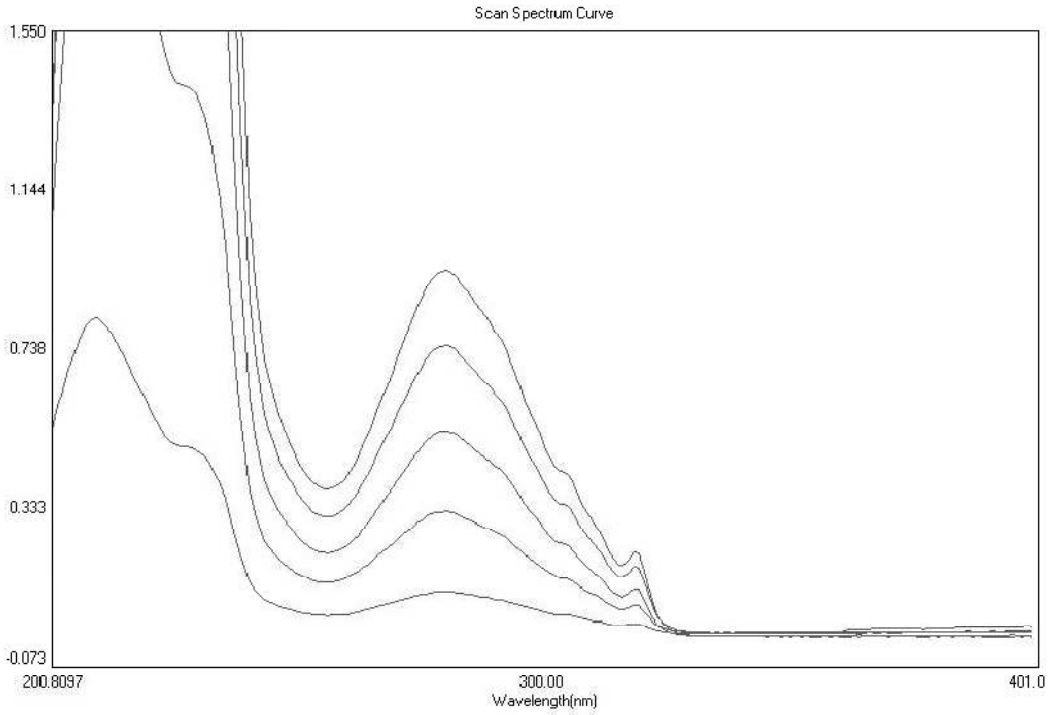

Fig. 3. Overlaid UV-spectra of naftopidil 25 µg ml–1 at 0 h time and 48 h time (thick line - 0 hour and dotted line - 48 hour)

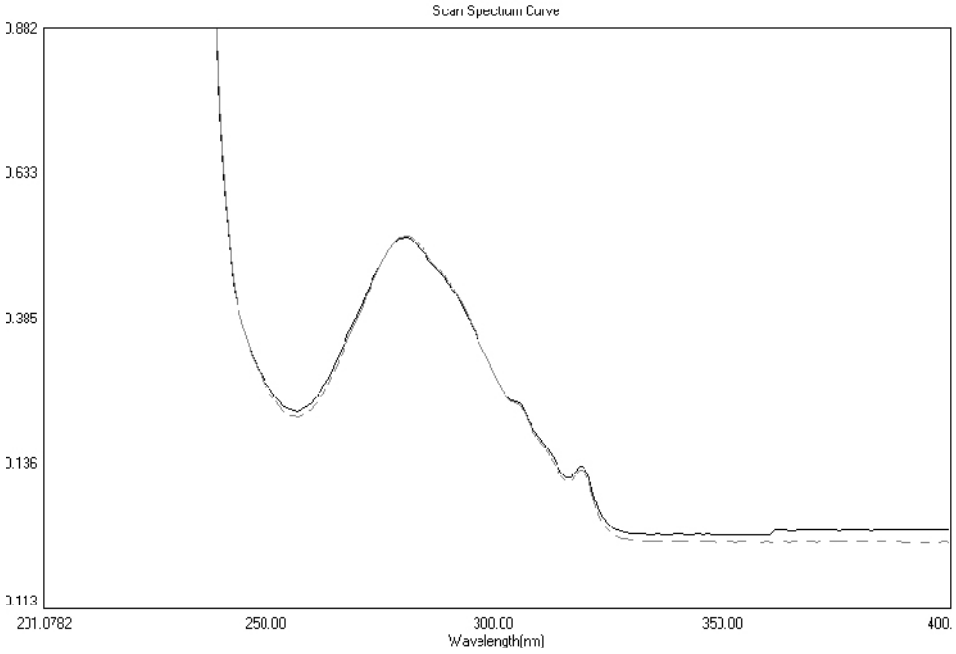

Fig. 4. Overlaid UV-spectra of naftopidil 10 µg ml–1 in bulk and tablet formulations (dotted lower line- nafodil tablet formulation, middle line- standard naftopidil in bulk, square block upper line- naftomax tablet formulation)

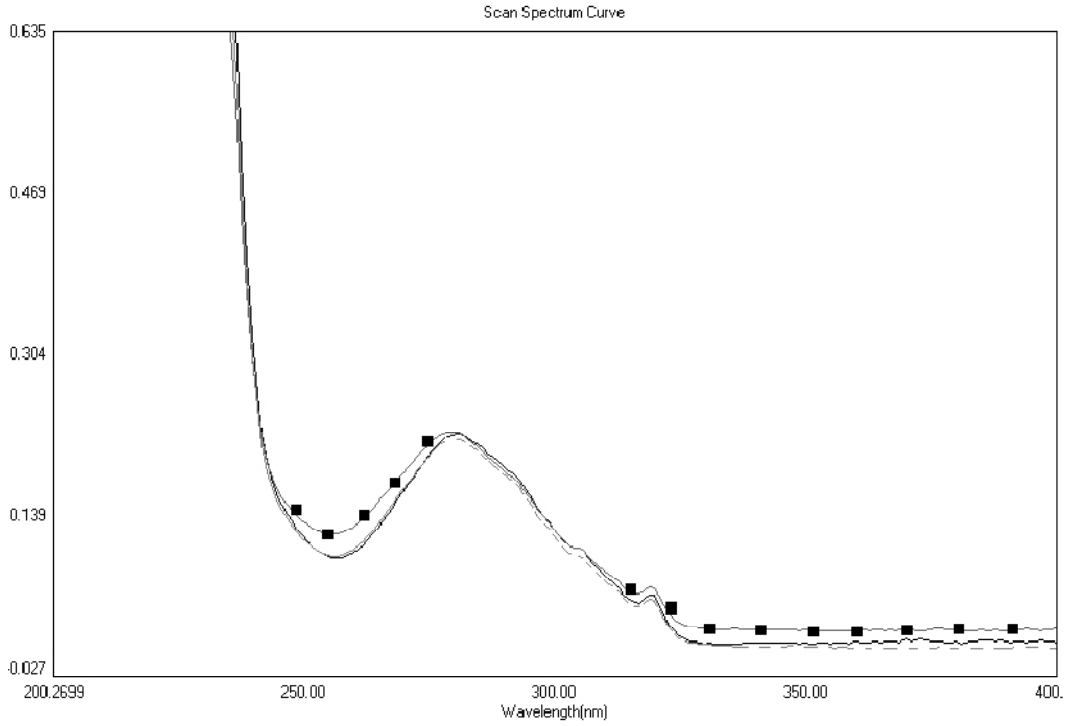

Table 1 Calibration data of the developed method (*N* = 9)

Drug concentration(µg ml–1)	Absorbance at 280 nm (±S.D)	% R.S.D.
5	0.1163 ± 0.0038	3.35
15	0.3238 ± 0.0051	1.57
25	0.5256 ± 0.0063	1.19
35	0.7483 ± 0.0086	1.15
45	0.9416 ± 0.0055	0.58

Table 2 Optical characteristics, statistical data and validation parameters for naftopidil (*N* = 9)

Parameters	Values
Apparent molar absorptivity (l mol−1 cm−1)	8.45 × 103
Slope (S.E. a)	0.0208 (6.68 × 10-5)
95% confidence limits of slope	0.02005; 0.02145
Intercept (S.E. a)	0.0123; (1.44 × 10-3)
95% confidence limits of intercept	-0.0077; 0.03242
Standard error of estimate	5.55 × 10-3
Regression coefficient (*r*2)	0.9997
Calculated *F*-value (critical *F*-value) b	0.00058 (1.5984)
Specificity and selectivity - *t*Cal (*t*Crit) c	0.98 (2.26)
Linearity (µg ml–1)	5 – 45
DL (µg ml–1)	0.68
QL (µg ml–1)	2.08
Robustness (mean % recovery ± S.D.)	100.29 ± 1.33

a Standard error of mean.
b Theoretical value of F based on one-way ANOVA test at P = 0.05 level of significance.
c tCal is calculated value and tCrit is theoretical value based on paired t-test at P = 0.05 level of significance.

Table 3 Accuracy and precision data from single stock solution (*N* = 9)

Level	Predicted Con. (µg ml–1)			Mean %Recovery(±S.D)	Accuarcy(%)
	Range	Mean(±S.D)	%R.S.D		
LC	9.88- 10.22	10.22±0.16	1.59	99.70±1.32	0.29
IC	29.84-30.17	29.89±0.23	0.76	100.35±0.94	-o.35
HC	39.81-40.17	39.90±0.26	0.65	100.25±0.61	-0.25

a Predicted concentration of naftopidil.
b Accuracy is given as, % relative error = [(predicted concentration – nominal concentration)/nominal concentration)] × 100.

Table 4 Standard addition method (*N* = 9)

Concentration of drug in formulations (µg ml-1)	Concentration of pure drug added (µg ml–1)	Total concentration of drug found (µg ml–1)	%Analytical recovery (± S.D.)
10	10	20.03	100.33 ± 1.00
10	20	30/02	100.12 ± 0.97
10	30	40.05	100.19 ± 0.29

Table 5 Results of intermediate precision study from single stock solution

Concentration (µg ml–1)	(Intra-day repeatability ± S.D.) % R.S.D.a (*N* = 9)			Inter-day repeatability % R.S.D. a (*N* = 27)
	Day 1	Day 2	Day 3	
10	0.2197 ± 0.0038 (1.69)	0.2214 ± 0.0017 (0.79)	0.2211 ± 0.0015 (0.66)	1.23
30	0.6342 ± 0.0050 (0.79)	0.6376 ± 0.0035 (0.56)	0.6655 ± 0.0014 (0.22)	0.52
40	0.8423 ± 0.0048 (0.57)	0.8442 ± 0.0011 (0.13)	0.8438 ± 0.0023 (0.27)	0.32

a Relative standard deviation.

Table 6 Estimation of naftopidil in tablet formulations (*N* = 5)

Commercial Products	Amount Found	% Assay
Naftomax (50mg)		
Mean ± S.D. (mg) *t*Cal (*t*Crit) a *F*Cal (FCrit) b	50.70 ± 0.35 0.10 (2.13) 0.21 (5.31)	101.39 ± 0.71
Naftodil (75mg)		
Mean ± S.D. (mg) *t*Cal (*t*Crit) a *F*Cal (FCrit) b	75.54 ± 0.86 0.44 (2.13) 0.29 (5.31)	100.72 ± 1.14

a tCal is calculated value and tCrit is theoretical value (at 4 d.f.) based on paired t-test at P = 0.05 level of significance.
b Theoretical value of F(1, 9) based on one-way ANOVA test at P = 0.05 level of significance.

CONCLUSION

The proposed UV spectrophotometric method is simple, sensitive, accurate, precise and cost effective and hence can be used for the routine analysis of naftopidil in bulk, tablet formulations and for dissolution studies.

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