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Original Research Article **Pharmaceutical Analysis**

Development and Validation of New Spectrophotometric Methods for the Quantitative Estimation of Naftopidil in Bulk and Pharmaceutical Formulation

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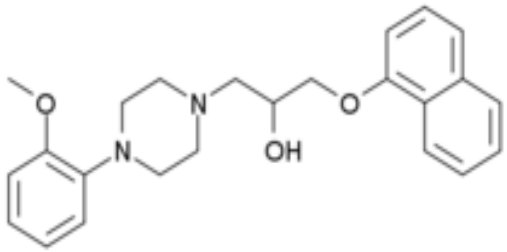
Abstract

Four simple, sensitive, precise and accurate spectrophotometric methods A, B, C and D have been developed for the quantitative estimation of Naftopidil. UV method (A) has been developed by using alcohol as the solvent. The wavelength of maximum absorbance selected was 269nm. The method was linear between the range of 2-10µg/ml with correlation coefficient of 0.9999. %RSD of precision was calculated and found to be 0.965. Colorimetric Methods B, C and D have been developed by oxidation followed by complex formation reaction using 1,10- phenanthroline, 2,2'-bipyridyl and Potassium ferricyanide respectively, using ferric chloride as oxidizing agent. Regression analysis for the methods was carried out and the correlation coefficient was found to be between the range of 0.99969-0.9999 depicting very good linear relationship between the concentration of the drug and corresponding absorbance values. % RSD of precision for the three colorimetric methods was calculated and found between the range of 0.4126-1.1354 showing that the methods are precise. Accuracy studies were carried out at three different levels and the results fall between the range of 99.89 to 99.96.

Keywords: Naftopidil, UV/ Visible spectrophotomety, oxidation followed by complex formation.

INTRODUCTION

Naftopidil



Chemical name: 1-[4-(2-methoxyphenyl)piperazin-1-yl]-3-(1-naphthyloxy)propan- 2-ol.

Category: Antihypertensive drug which acts as a selective α -1 adrenergic receptor antagonist or alpha blocker.

Naftopidil is a α1-adrenergic receptor antagonist (α1-blocker) used to treat lower urinary tract symptoms (LUTS) suggestive of benign prostatic hyperplasia (BPH) [1]. Among the various heterocyclic compounds the drugs used in the treatment of BPH are Silodosin, Naftopidil, Tamsulosin [2-4], Doxazosin [5, 6], Prazosin [7], Indoramine, Alfuzosin etc [8, 9]. Different from tamsulosin hydrochloride, in that it has higher and extremely higher affinity respectively, for the α1A-adrenergic receptor subtype than for the α1D type, Naftopidil has distinct characteristics because it has a three times greater affinity for the α1D-adrenergic receptor subtype than for the α1A subtype. Different from tamsulosin and Silodosin, which have higher and extremely higher affinity for the α1A-AR subtype than for the α1D-AR subtype, respectively, Naftopidil has distinct characteristics because it has threefold affinity for the α1D subtype than for the α1A-AR subtype.[121, 122] Since the tissue of BPH shows nine- and threefold increased expression of mRNA of α1A and α1D-AR subtypes, respectively, compared to normal prostatic tissue,[123] it has been speculated that not only α1A but also α1D-AR contributes to contraction of prostatic smooth muscle. Tamsulosin and Naftopidil were more

effective in patients with dominant expression of α1a and α1d, respectively. Thus, in approximately half of the patients having LUTS/BPH, the efficacy of the α1D/α1A blocker Naftopidil may be more promising than that of the α1A/α1D blocker tamsulosin. Naftopidil had a similar short-term efficacy and adverse-effect profile compared to low-dose tamsulosin, and better efficacy than phytotherapy.

One spectrophotometric [10], one RP-HPLC [11] and chiral HPLC [12] method have been developed for the quantitative estimation of Naftopidil which drives us for the development of simple spectrophotometric methods for the quantitative estimation of Naftopidil in bulk as well as pharmaceutical formulation.

MATERIALS AND METHODS

In the present investigation Systronics 2203 double beam UV/ Visible Spectrophotometer with 1 cm matched quartz cells are used for the quantitative estimation of Naftopidil by UV/ Visible spectrophotometric methods.

Reagents and chemicals: All the reagents and chemicals required for the development of spectrophotometric methods were of analytical grade and were procured from various standard pharmaceutical companies.

Experimental:
Ferric chloride (aqueous O.25%w/v, aqueous 0.01 M)
1,10- phenanthroline (aqueous 0.2%w/v)**,**
2,2'- bipyridyl (aqueous 0.2%w/v),
Potassium ferricyanide (aqueous 0.025%w/v),
Distilled water
Distilled Alcohol

Preparation of standard stock solution of Naftopidil:

Standard stock solution was prepared by dissolving 100mg of Naftopidil in sufficient amount of alcohol in a 100ml volumetric flask dissolved properly and volume was made up to the mark with alcohol (1000 µg/ml).

Preparation of Working Stock Solution (WSS) of Naftopidil for the Development of UV method:

10ml of standard stock solution was pipetted out in a 100 ml volumetric flask and volume was made up to the mark with a mixture of water: alcohol 50: 50% v/v (100µg/ml).

Method A: Five different aliquots of Naftopidil ranging from 2-10 µg/ml were prepared by pipetting 0.2, 0.4, 0.6, 0.8 and 1.0 ml WSS in different 10 ml volumetric flasks and the volume was made up to the mark by using water : alcohol (50 : 50% v/v) mixture.

Working Stock Solution (WSS alc) for the Development of Visible Spectrophotometric Methods:

From the standard stock (1000 µg/ml) as described earlier, about 10 ml solution was taken in a 100 ml volumetric flask and the volume was made up to the mark with alcohol (100µg/ml). Three colorimetric methods were developed using this working stock in the present investigation for the estimation of Naftopidil by using 1, 10- phenanthroline (method B), 2,2'-bipyridyl (method C) and potassium ferricyanide (method D).

Method B: Five aliquots were prepared by taking 0.1, 0.2, 0.3, 0.4 and 0.5 ml WSS (alc) in five different 10 ml volumetric flasks. To each of the flask added 0.5 ml of 0.25% aqueous $FeCl_3$ solution and 1 ml of 0.2% aqueous 1, 10- phenanthroline solution. The flasks were heated at 60^{o}C for 20 min. Allow to cool and volume was made up to the mark with distilled water.

Method C: Five aliquots of the drug were prepared by pipetting 0.1, 0.2, 0.3, 0.4 and 0.5 ml WSS (alc) in five different 10 ml volumetric flasks. To each of the flask added 0.5 ml of 0.25% aqueous $FeCl_3$ solution and 1.5 ml of 0.2% aqueous 2,2'-bipyridyl solution. The flasks were heated at 80^{o}C for 20 min, allowed to cool at room temperature and volume was made up to the mark with distilled water.

Method D: Potassium ferricyanide was used for the colorimetric estimation of Naftopidil in presence of ferric chloride. Aliquots were prepared by transferring 0.1, 0.2, 0.3, 0.4 and 0.5 ml WSS (alc) in different 10 ml volumetric flasks. To each of the flask about 0.5 ml of 0.01 M aqueous ferric chloride solution and 1.5 ml of 0.025% w/v aqueous potassium ferricyanide solution was added respectively. The solutions were allowed to stand for 20 min with occasional shaking for the completion of reaction and then volume was made up to the mark with distilled water.

Parameters fixation:

In developing these methods, a systematic study of the effects of various relevant parameters in the methods concerned were under taken by changing one variable at a time (OVAT) and controlling all other parameter to get the maximum colour development, reproducibility and reagent concentration. The effect of various parameters such as concentration, volume of reagents, order of addition of reagents and solvent for final dilution were studied by means of control experiments varying one parameter at a time.

RESULTS AND DISCUSSION

As per the ICH guidelines the method validation parameters checked for the developed methods were linearity, precision, accuracy, ruggedness, LOD, LOQ and robustness.

Selection of λ max:

Appropriate wavelengths of maximum absorbance for the developed methods were selected by scanning the middle concentration solution from the Beer's law range. The λmax for the UV method of Naftopidil was found to be 230 nm, whereas the absorption maxima for the colorimetric methods were found to be 509, 520 and 745nm for method B, C and D respectively

Linearity range:

For linearity studies graphs were plotted using obtained data for the proposed methods and results were given in table no 1.

Optical Characteristics:

The absorbance at appropriate wavelengths of a set of solutions containing different amounts of Naftopidil and specified amount of reagents (as described in the recommended procedures) were noted against corresponding reagent blank for different methods developed. The Beer's law plot for the obtained data was illustrated graphically. Least square regression analysis was carried out for the slope, intercept and correlation coefficient for each method. Beer's law limits, molar absorptivity, Sandell's sensitivity were calculated and the results are summarized in table no1.

Precision:

Six aliquots (middle concentration) were prepared according to the prescribed methods and absorbance was measured. RSD was calculated for the same and was found to be within the prescribed limit. Data was provided in table no 1.

Limit of Detection:

To determine LOD, middle concentration solution of the linearity range for each of the method was selected. The data obtained was suitably processed to obtain the values. The results are given in Table no 1.

Limit of Quantification:

This is the minimal quantity of the drug which can be estimated by the proposed method. LOQ for each of the methods were calculated by using the middle concentration of the linearity range for the respective method and the results are tabulated in Table no 1.

Analysis of formulations:

Commercially available dosage form of the drug was analyzed by the proposed methods and the results are shown in Table 2.

Accuracy:

This shows high accuracy of the proposed methods results are tabulated in Table 3-6.

Colour Stability:

To study the stability of the developed colour for proposed methods, middle concentration of linearity range was selected. Colour stability was measured against time. It was found that the developed chromogens were stable for suitably longer duration.

Results for the colour stability studies for the proposed methods were provided in Table no 7-9.

Table 1: Optical Characteristic and Regression Analysis Data of Naftopidil

Sr. No	Optical Characteristic	Method A	Method B	Method C	Method D
1.	$\lambda_{max.}$	230	509	520	745
2.	**Linearity range**	2-10	1-5	1-5	1-5
3.	**Sandell's sensitivity µg/cm^2**	0.07	0.006	0.009	0.008
4.	**Molar absorptivity**	$3.384374x10^4$	$6.7274x10^4$	$4.388099x10^4$	$5.89528x10^4$
5.	**Correlation co-efficient (r)**	0.9999	0.9998	0.99997	0.99969
6.	**Slope (b)**	0.08705	0.1661	0.1095	0.1456
7.	**Intercept (a)**	-0.0091	0.0267	0.0121	0.0234
8.	**RSD of Precision**	0.9649978	0.41259	1.13540	0.592788
9.	**Average recovery**	99.93±0.17	99.965±0.095	99.89±0.212	99.90±0.0907
10.	**Colour stability (min)**	----------	105	120	90
11.	**LOD (µg/ml)**	0.18941	0.0435	0.1154	0.060
12.	**LOQ (µg/ml)**	0.57399	0.132	0.349745	0.183
13.	**Percentage assay of formulation (Mean± SD)**	100.03±0.19	99.92±0.582	99.87±0.519	100.05 ± 0.18
14.	**Range of error**				
	0.05 confidence limit	$5.3750842x\ 10^{-3}$	$2.356815\ X\ 10^{-3}$	$4.119748\ x\ 10^{-3}$	$2.86638\ x\ 10^{-3}$
	0.01 confidence limit	$7.1375407x\ 10^{-3}$	$3.129600\ X10^{-3}$	$5.470588\ x\ 10^{-3}$	$3.80625\ x\ 10^{-3}$
15.	**Standard error of method**	$2.03988x\ 10^{-3}$	$8.94427\ X\ 10^{-4}$	$1.56347\ x\ 10^{-3}$	$1.0878\ x\ 10^{-3}$

Table 2: Analysis of Naftopidil Tablet Formulation by the Proposed methods with Statistical Evaluation (n=6)*

METHOD	Label Claim (mg)	Reference Method Mean	%Drug estimated Mean*± SD	%RSD	SEM
A	50	99.898	100.03±0.19	0.1976	0.0807
B	50	99.898	99.92±0.58165	0.58211	0.23745
C	50	99.89	99.87±0.51919	0.51986	0.21196
D	50	99.898	100.05±0.18	0.18126	0.0740

*Mean of 6 determinations
**reference method

Table 3: Recovery Studies of Naftopidil Using the Proposed Method with Statistical Evaluation (n=3)*(Method A)

Concentration of formulation (%)	Label claim (mg)	Pure drug spiked (mg)	Statistical results			SEM*
			Mean*	SD	%RSD	
50	50	25	99.82	0.433	0.4344	0.1770
100	50	50	99.84	0.497	0.4978	0.2029
150	50	75	100.14	0.358	0.3578	0.1462

*Mean of 3 determinations

Table 4: Recovery Studies of Naftopidil Using the Proposed Method with Statistical Evaluation (n=3)* (Method B)

Concentration of formulation (%)	Label claim (mg)	Pure drug spiked (mg)	Statistical results			SEM*
			Mean*	SD	%RSD	
50	50	25	99.855	0.45302	0.4536	0.184945
100	50	50	100.015	0.12437	0.12435	0.050777
150	50	75	100.025	0.1505	0.05052	0.061468

*Mean of 3 determinations

Table 5: Recovery Studies of Naftopidil Using the Proposed Method with Statistical Evaluation (n=3)* (Method C)

Concentration of formulation (%)	Label claim (mg)	Pure drug spiked (mg)	Statistical results			SEM*
			Mean*	SD	%RSD	
50	50	25	99.653	0.76064	0.7632	0.31053
100	50	50	99.98	0.34449	0.34456	0.140641
150	50	75	100.05	0.263058	0.26292	0.107393

*Mean of 3 determinations

Table 6: Recovery Studies of Naftopidil Using the Proposed Method with Statistical Evaluation (n=3)* (Method D)

Concentration of formulation (%)	Label claim (mg)	Pure drug spiked (mg)	Statistical results			SEM*
			Mean*	SD	%RSD	
50	50	25	99.82	0.5348	0.5357	0.2183
100	50	50	99.98	0.1804	0.1805	0.0736
150	50	75	100.00	0.1271	0.1271	0.0519

*Mean of 3 determinations

Colour Stability Studies of Naftopidil for

Table 7: (Method B)

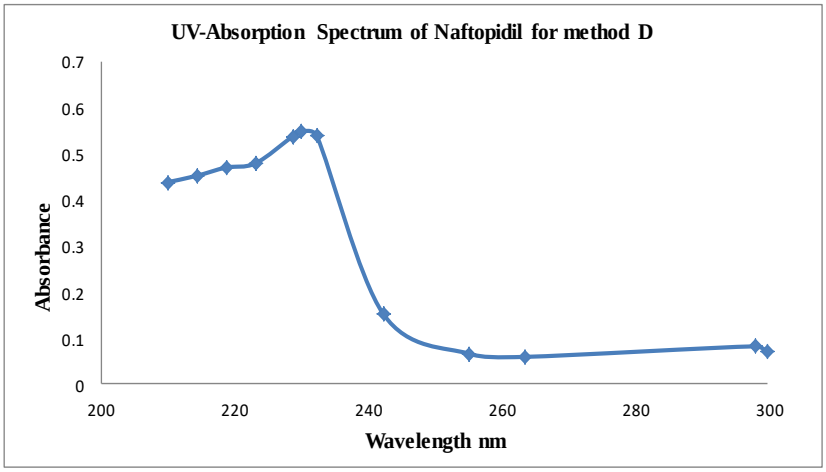
Concentration	**3µg/ml**										
Time(min)	10	20	30	40	50	60	70	80	90	100	110
Absorbance	0.532	0.532	0.533	0.532	0.532	0.532	0.532	0.531	0.532	0.532	0.530

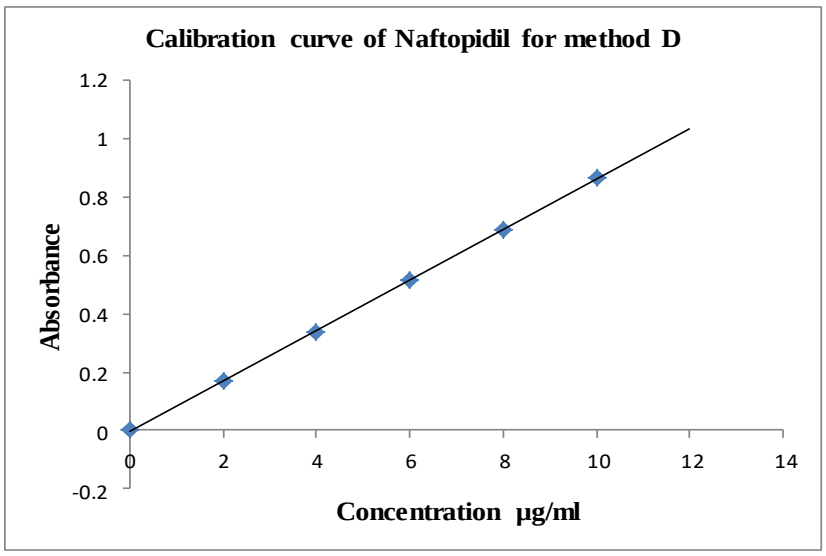
Table 8: (Method C)

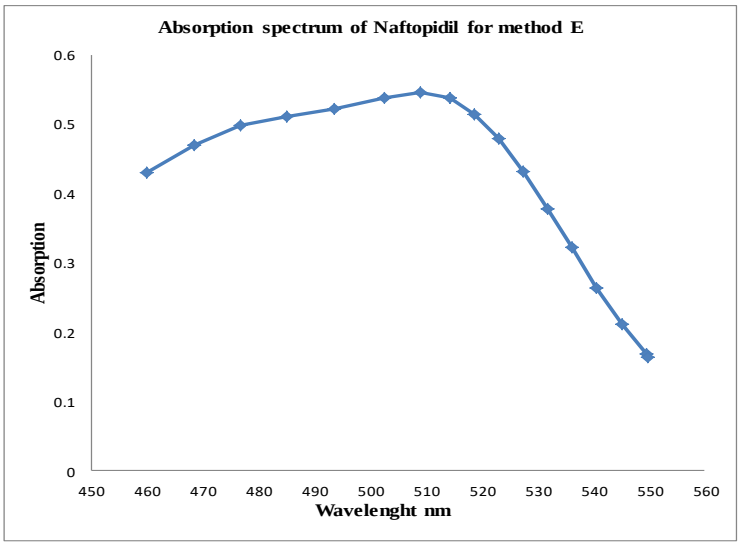
Concentration	**3µg/ml**											
Time(min)	10	20	30	40	50	60	70	80	90	100	110	120
Absorbance	0.342	0.342	0.342	0.342	0.341	0.342	0.342	0.342	0.342	0.343	0.342	0.341

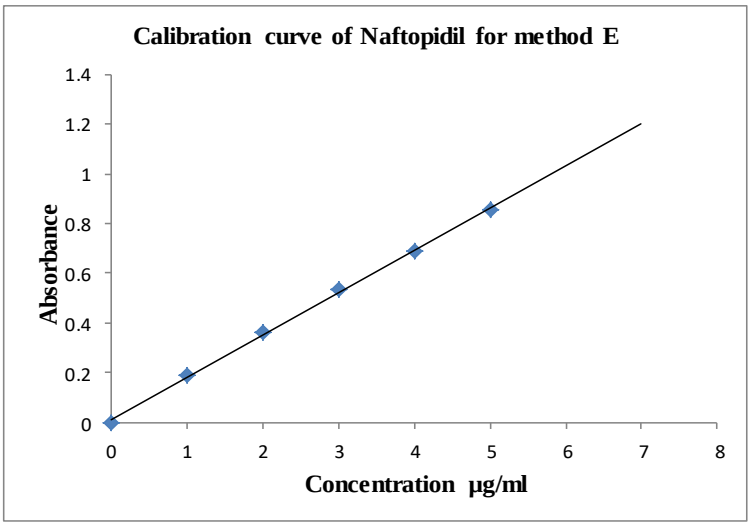
Table 9: (Method D)

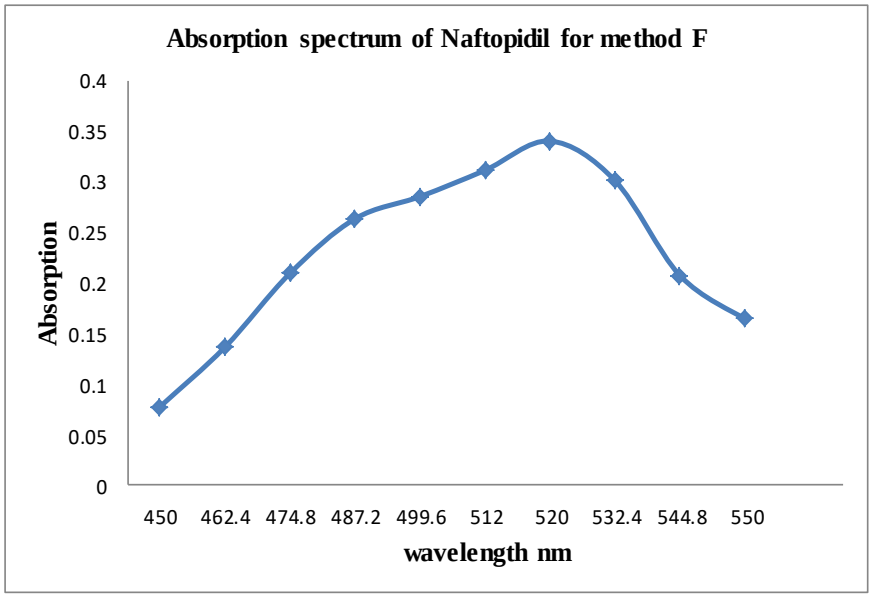
Concentration	**3µg/ml**								
Time(min)	10	20	30	40	50	60	70	80	90
Absorbance	0.451	0.451	0.451	0.452	0.451	0.450	0.451	0.450	0.450

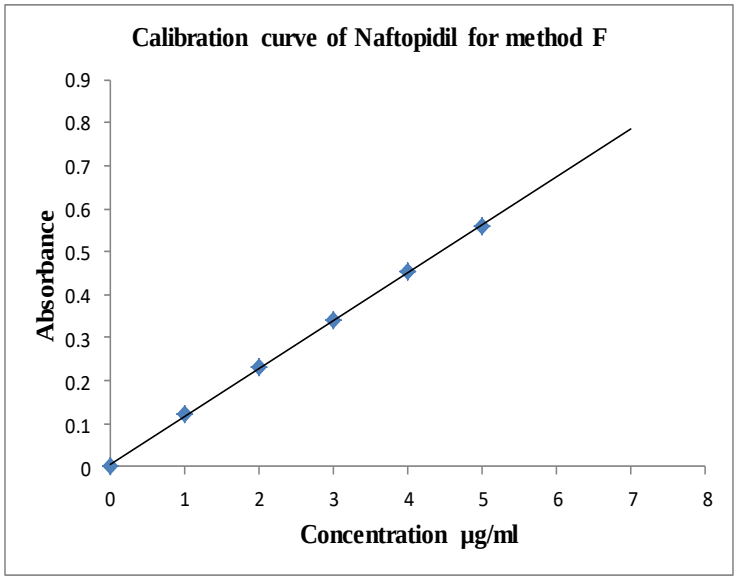


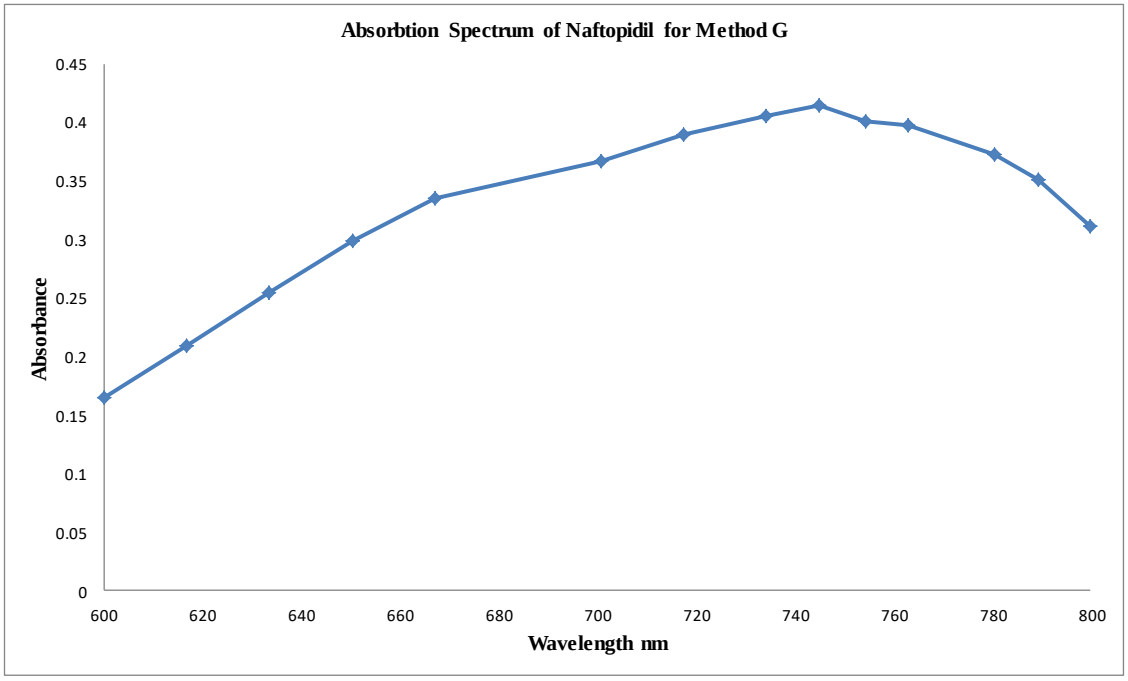


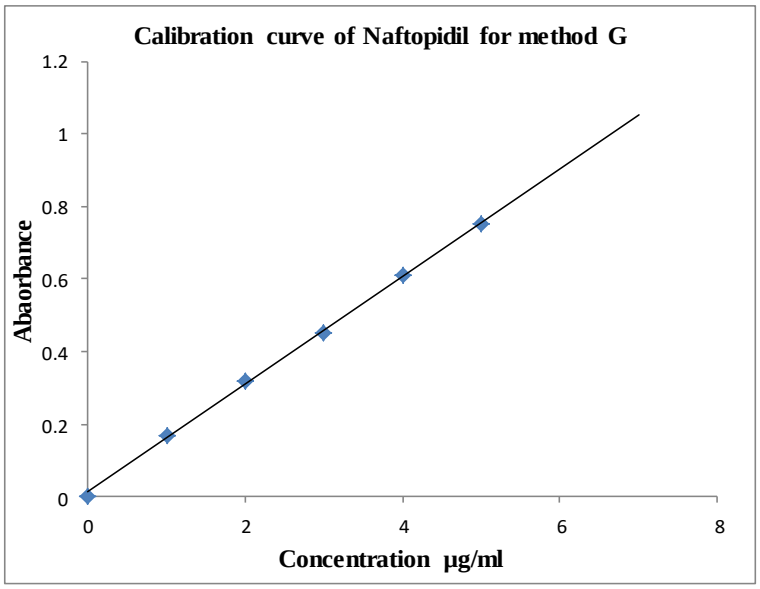



Calibration curve of Naftopidil for method E
Absorbance
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0 1 2 3 4 5 6 7 8
Concentration µg/ml


Absorption spectrum of Naftopidil for method F
Absorption
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
450 462.4 474.8 487.2 499.6 512 520 532.4 544.8 550
wavelength nm


Calibration curve of Naftopidil for method F
Absorbance
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0 1 2 3 4 5 6 7 8
Concentration µg/ml





CONCLUSION

Four simple, sensitive, precise and accurate spectrophotometric methods have been developed for the quantitative estimation of Naftopidil.

Precision of each one among the proposed spectrophotometric methods were ascertained separately from the absorbance values obtained by actual determination of a fixed amount of drug in final solution. The percent relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated.

Commercial formulation of Naftopidil was successfully analyzed by the proposed methods. The results obtained by developed methods were compared with reported UV method (reference method) and found in good agreement with the labelled amount in formulation. This shows applicability of the proposed methods for the routine analysis of the drug.

Recovery studies were carried out at three different levels i.e., 50%, 100% and 150%, of label claim following standard addition method. Results were statistically calculated. The results obtained are within the acceptance criteria.

- UV method has been developed by using alcohol as the solvent. The wavelength of maximum absorbance selected was 230nm. The method was linear between the range of 2-10µg/ml with correlation coefficient of 0.9999. % RSD of

precision was calculated and was found to be 0.965.

- Methods B, C and D have been developed by oxidation followed by complex formation reaction by using 1,10- phenanthroline, 2,2'- bipyridyl and Potassium ferricyanide. Ferric chloride was used as the oxidizing agent.
- Regression analysis for the methods were carried out and the correlation coefficient for the methods were found to be between the range of 0.9997-0.9999 depicting very good linear relationship between the concentration of the drug and absorbance values obtained.
- % RSD was calculated to determine the precision of the developed methods and it was found to lies between the range of 0.4126-1.28 showing good precision of the methods. Lower range of % for the method B depicts that it is more precise amongst the methods developed for the quantitative estimation of Naftopidil.

Accuracy studies for all four methods developed for the quantitative estimation of Naftopidil were carried out at three different levels and the results fall in the range of 99.08 to 100.65 which lies within the acceptable limit.

- LOD and LOQ for the proposed methods were calculated and were found to be in the range of 0.01-0.189 and 0.03-0.574 respectively. Method B was found to be most sensitive method for the estimation of Naftopidil with minimum LOD and LOQ values.
- Assay of formulation was carried out by following the proposed methods and the results were found to be in the range of 99.87-100.05.

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