DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF NAFTOPIDIL AS API AND IN TABLET DOSAGE FORM

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ABSTRACT

A reversed phase High Performance Liquid Chromatographic method for the estimation of Naftopidil has been developed. The drug was separated on ODS column using methanol and water as mobile phase in ratio of 80:20 v/v at flow rate of 0.8 ml/min. Components are detected simultaneously at 280 nm using UV detector. The detection limits for Naftopidil was 0.0327 μg/ml where as the quantitation limits was 0.0991 μg/ml. Linearity range was established in range of 2–12 μg/ml for Naftopidil. Recovery of the added Naftopidil standard mixture in tablet solution was found 99.81 ± 0.384 with Relative standard deviation (n=3) of 0.384 %. The proposed method has been applied to the determination of Naftopidil in commercial products. The results obtained by methods were in good agreement of true values. The proposed method is simple, accurate, reproducible and suitable for routine analysis.

KEYWORDS: Naftopidil, RP HPLC, ICH

INTRODUCTION

Introduction to High Performance Liquid Chromatography.\textsuperscript{[1-8]}

HPLC is a chromatographic technique used to separate the components in a mixture, to identify each component, and to quantify each component. It relies on pumps to pass a
pressurized liquid and a sample mixture through a column filled with a sorbent, leading to the separation of the sample components.

**Modes of separation in HPLC**

They are normal phase mode, reversed phase mode, reverse phase ion pair chromatography, affinity chromatography and size exclusion chromatography (gel permeation and gel filtration chromatography).

1. **Normal phase mode**: In this mode, the stationary phase is polar and the mobile phase is non polar in nature. In this technique, non polar compounds travel faster and are eluted first. This is because of the lower affinity between the non polar compounds and the stationary phase. Polar compounds are retained for longer times because of their higher affinity with the stationary phase. These compounds, therefore take more times to elute. Normal phase mode of separation is therefore, not generally used for pharmaceutical applications because most of the drug molecules are polar in nature and hence take longer time to elute.

2. **Reversed phase mode**: This is most popular mode for analytical and preparative separations of compound of interest in chemical, biological, pharmaceutical, food and biomedical sciences. In this mode, the stationary phase is non polar hydrophobic packing with octyl or octadecyl functional group bonded to silica gel and the mobile phase is polar solvent. An aqueous mobile phase allows the use of secondary solute chemical equilibrium (such as ionization control, ion suppression, ion pairing and complexation) to control retention and selectivity. The polar compound gets eluted first in this mode and non polar compounds are retained for longer time. As most of the drugs and pharmaceuticals are polar in nature, they are not retained for longer times and hence elute faster. The different columns used are octadecylsilane (ODS) or C₁₈, C₈, C₄, etc., (in the order of increasing polarity of the stationary phase).

3. **Ion pair chromatography**: This method issued for the separation of ionic compounds and this method can also substitute for ion exchange chromatography. Strong acidic and basic compounds may be separated by reversed phase mode by forming ion pairs (columbic association species formed between two ions of opposite electric charge) with suitable counter ions. This technique is referred to as reversed phase ion pair chromatography or soap chromatography.
4. **Affinity chromatography:** In this method, stationary phase contains specific groups of molecules which can absorb the sample if certain strict and charge related conditions are satisfied. This technique can be used to isolate proteins, enzymes as well as antibodies from complex mixtures.

5. **Size exclusion chromatography:** In this method molecules are separated according to their molecular mass. Largest molecules are eluted first and the smallest molecules last. This method is generally used when a mixture contains compounds with a molecular mass difference of at least 10%. This mode can be further subdivided into gel permeation chromatography (with organic solvents) and gel filtration chromatography (with aqueous solvents).

**Various components of HPLC**

![HPLC equipment diagram](image)

**Fig 1: A schematic diagram of HPLC equipment**

1. A solvent delivery system, including pump,
2. Sample injection system,
3. A chromatographic column,
4. A detector,
5. A strip chart recorder,
6. Data handling device and microprocessor control.

**Analytical method validation.**[^9-11]

Validation is an act of proving that when any procedure, process, equipment, material, activity or system is performed as expected under given set of conditions then it should give the required accuracy, precision, sensitivity, ruggedness to the method/system etc. When extended to an analytical procedure, depending upon the application, it means that a method...
works reproducibly, when carried out by same or different persons, in same or different laboratories, using different reagents, different equipments, etc.

The various validation parameters used as per ICH guideline are:

1. Accuracy,
2. Precision (intraday and inter day precision, Repeatability and Reproducibility)
3. Linearity
4. Range
5. Limit of Detection (LOD) and Limit of Quantitation (LOQ)
6. Selectivity/ Specificity
7. Robustness/ Ruggedness

**Introduction of Naftopidil.**[12-17]

Naftopidil is an α1-adrenergic receptor antagonist (α1-blocker) used to treat lower urinary tract symptoms (LUTS) suggestive of benign prostatic hyperplasia (BPH). 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.

**STRUCTURE**

![Naftopidil Structure](image)

**IUPAC Name**

4-(2-Methoxyphenyl)-alpha-((1-naphthalenloxy)methyl)-1-pioerazineethanol dichloride;(+)-1-(4-(2Methoxyphenyl)piperazinyl) -3- (1-naphthyloxy)propan-2-ol dichloride.

**Molecular formula** $C_{24}H_{28}N_{2}O_{3}$

**Molecular Weight** 392.5 g/mol

**Pharmacodynamics**

Naftopidil is a novel alpha-1 adrenoreceptor blocker. The phenylpiperazine derivative competitively inhibits prazosin-binding prostatic membrane receptors. The selective action...
against adrenoreceptors leads to reduced blood pressure and prostate pressure. Naftopidil reduces the bladder outlet obstruction in benign prostate hyperplasia patients.

**Mechanism of Action**

Benign prostatic hyperplasia (BPH) is common in men above a certain age throughout the world. Alpha1-adrenoceptor antagonists is widely used as a conservative treatment to relieve bladder outlet obstruction due to benign prostatic enlargement. Naftopidil is a newly synthesized alpha1-blocker that has been found to be effective in the treatment of BPH. This drug is highly selective for the Alpha1A-, and Alpha1D-adrenoceptor subtypes, with an affinity for the Alpha1D-adrenoceptor that is 3- and 17-fold higher than that for the Alpha1A- and Alpha1B-adrenoceptors.

**Pharmacokinetics** In healthy adult volunteers, after once oral administration of Naftopidil alone with 25mg, 50mg and 100mg, the Tmax is 0.45±0.21, 0.75±0.71, 0.65±0.22 hours respectively, and the Cmax is 39.3±10.3, 70.1±32.9, 134.8±55.8 mg/ml respectively, while the half life is 15.2±4.7, 10.3±4.1, 20.1±13.7 hours. Once oral administration of 50mg after meal at a frequency of twice a day, the plasma concentration reaches plate phase after four dosages. Within 24 hours, parent drug eliminated in urine is less than 0.01%. The main metabolites are Glucuronide conjugates hydroxide and methoxyphenyl. In healthy adult volunteers, Tmax of before and after meal is 0.75 and 2.20 hours respectively. Peak concentration and elimination half life have no significant changes to suggest that food has little influence on Naftopidil's absorption. In healthy adult volunteers, the serum protein binding rate of this product is 98.5% when 100mg of the drug is administered before meal. Indications Naftopidil is indicated for the relief of the signs and symptoms of bladder outlet obstruction and dysuria due to benign prostatic enlargement.

**Adverse Reactions** Common adverse reactions are shown below: Dizziness (0.98%), headache (0.33%), tinnitus (0.33%), constipation (0.33%), uneven feelings of stomach (0.33%), edema (0.33%), coldness (0.33%), laboratory test findings: ALT increase (1.53%), aspartate amino-transferase increase (1.34%).

**Contraindications** Naftopidil is contraindicated for patients with allergic reactions to any ingredients. Precautions General Antihypertensive agents and diuretics in combination with Naftopidil will have additional effects to cause hypotension. Therefore, prescription of such combination should reduce the dosage of Naftopidil. Patients below should use this drug with caution:
1. Patients with severe cardiovascular diseases or hepatic impairment should use Naftopidil with caution.
2. Patients doing height work or driving.
3. Patients with hypotension or using antihypertensive agents.
4. Orthostatic hypotension is possible during administration, so it is suggested that patients take the medication before sleep

**Pediatric use Children** is contraindicated for this drug.

**Geriatric use** Naftopidil is eliminated mainly via hepatic metabolism. Elderly men usually experienced declined hepatic function that makes drug metabolism much slower and plasma concentration consistently higher than normal. So geriatric use should consider dose reduction.

**Dosage and Administration Oral administration.** Usually initial dosage is 25mg once-a-day before sleep. Dosage adjustment depends on the doctor's decision according to clinical effects. Maximum daily dosage is 75mg. Elderly patients should begin with lower initial dose such as 12.5mg with caution.

**MATERIALS AND METHODS.**[18-25]

**Chemicals and reagents**
Naftopidil bulk drug was made available from Cadila Healthcare Pvt.Ltd Ahmedabad, Gujrat. Orthophosphoric acid, methanol, Perchlorate buffer, Acetonitrile were obtained from Merck.. All chemicals and reagent used were of HPLC grade, Milli-Q-water was used throughout the experiment. Equipments: The Waters HPLC system with a UV or photo diode array detector was used for method development and validation. The output signal was monitored and processed by using Empower software. Chromatographic condition: The mobile phase used Methanol and Water in the gradient mode employing at a flow rate of 0.8 ml/min. The analytical column used Inertsil ODS C18 (4.0 x 250mm, 6 detection was carried out at a wavelength of 280 nm for a run time of 8 min. Diluent used as Methanol.

**Preparation of standard stock solution of Naftopidil**
10 mg of Naftopidil was weighed accurately and transferred into 10 ml volumetric flask. About 10 ml of HPLC grade Methanol was added and sonicated to dissolve. The volume was made up to the mark with same solvent to form 1000μg/ml solution. 1.0 ml of the stock solution was further diluted in a 10 ml volumetric flask with same solvent to form 100 μg/ml
solutions and 1.0 ml of 100 μg/ml was diluted further up to 10 ml with same solvent. The final solution contained about 10 μg/ml of Naftopidil. The solution was filtered through the 0.45 μm membrane filter and degassed under ultrasonic bath prior to use.

**Preparation of Sample Solution of Naftopidil**

One tablet was weighed, powdered and then the weight was transferred into a 100mL volumetric flask, 50mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluent

**RESULT & DISCUSSION**

Chromatographic parameters were preliminary optimized to develop RP HPLC method for estimation of Naftopidil with short analysis time 8 min, and accepted resolution (>2). The isoabsptive point of Naftopidil was 280 nm. In order to identify a suitable organic modifier, various compositions of methanol, water and acetonitrile were tested along with different buffer. Different columns like Inertsil and Inspire columns were tried. Resolution and peak tailing were the measure problem while we are during development of method. Resolution and peak separation were very less when we are using one mobile phase, to increase the resolution and better peak separation of methanol and water were used in isocratic mode. Finally, separation for the determination Naftopidil was carried out by isocratic elution with a flow rate of 0.8 ml/min at 280 nm using inertsil (ODS 250 x 4.6 mm) The standard chromatogram shown in fig 2.

**Optimized Method:** Drug was eluted with good resolution, retention time all the parameters like Plate count and Tailing factor were within the limits.

**Mobile phase**

Methanol and Water taken in the ratio 80:20.

**Chromatographic conditions**

**Flow Rate.** 0.8ml/min  
**Column.** ODS (215x4.6mm)  
**Detector Wavelength.** 280nm  
**Column Temperature.** 30°C  
**Injection Volume.** 10uL
Run Time. 10min

Solvent System. Methanol : Water (80:20)

Fig: 2 Optimized chromatogram of Naftopidil

Table: 1 System suitability parameter of RP-HPLC method

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameters (n=5)</th>
<th>Naftopidil</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Retention Time (min)</td>
<td>2.4</td>
</tr>
<tr>
<td>2.</td>
<td>Theoretical Plates</td>
<td>5861</td>
</tr>
<tr>
<td>3.</td>
<td>Asymmetry</td>
<td>1.29</td>
</tr>
</tbody>
</table>

Method Validation
The above method was validated according to ICH guidelines to establish the performance characteristics of a method (expressed in terms of analytical parameters) to meet the requirements for the intended application of the method.

Specificity
Preparation and running of Naftopidil
The solution of Naftopidil was prepared. Accurately weighed 10 mg of Naftopidil was transferred to 100 ml of volumetric flask and 100 ml of methanol was added to it. 1 ml of this solution was taken in 100 ml flask and 50 ml of methanol was added common excipients used in tablet formulation such as 8% starch, 7% magnesium stearate, 1% talc and remaining lactose (for 100 μg/ml) were added in this solution and were sonicated for 20 minutes. Then solution was filtered through membrane filter and volume was made up to the 100 ml with solvent system and degassed under ultrasonic bath prior to use. The solution was then injected into the HPLC system. The chromatogram obtained of synthetic mixture is shown in figure 3.
Fig 3: Chromatogram for formulated synthetic tablet mixture

No peaks were found at the retention time of Naftopidil. The retention time were found to be 2.4 min. Specificity studies indicated that the excipients did not interfere with the analysis.

**Linearity**

Linearity range was found to be 2-12 µg/ml for Naftopidil. The correlation coefficient was found to be 0.999 which showed good linearity between ranges. The slope was found to be 16414 and intercept was found to be -2295.5.

**Preparation of calibration curve of standard API**

The standard solutions of Naftopidil in the concentration range of 2µg/ml to 12 µg/ml were obtained by transferring 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml from stock solution 100 µg/ml to the series of six volumetric flasks of 10 ml. The volumes in each volumetric flask were made up to the mark. The solutions were filtered, degassed and were injected into column. The run time was 10 min and the peak areas were measured. The calibration data are shown in table no. 2 and calibration curve is shown in figure4.

**Table 2: Calibration curve data for Naftopidil**

<table>
<thead>
<tr>
<th>S. no</th>
<th>Concentration (µg/ml)</th>
<th>Area* (µv* sec)</th>
<th>SD</th>
<th>R.SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>2</td>
<td>32448</td>
<td>1005.74</td>
<td>3.10</td>
</tr>
<tr>
<td>2.</td>
<td>4</td>
<td>63120</td>
<td>7660.29</td>
<td>12.13</td>
</tr>
<tr>
<td>3.</td>
<td>6</td>
<td>93344</td>
<td>1130.41</td>
<td>12.10</td>
</tr>
<tr>
<td>4.</td>
<td>8</td>
<td>129792</td>
<td>4840.67</td>
<td>3.73</td>
</tr>
<tr>
<td>5.</td>
<td>10</td>
<td>160240</td>
<td>9670.16</td>
<td>6.03</td>
</tr>
<tr>
<td>6.</td>
<td>12</td>
<td>196688</td>
<td>3760.88</td>
<td>1.91</td>
</tr>
</tbody>
</table>

*Mean of three triplicate determinations; µg/ml: - microgram per milliliter; µv*sec:- microvolt sec
Fig 4: Calibration Curve of Naftopidil

Range
Range of an analytical method is the interval between the upper and lower levels. It includes working range, linearity range and target range and target concentration.

1. **Working range:** It begins from limit of quantitation to the maximum concentration used for the development of the analytical method. For the developed method, the working range was found to be 0.342 to 20 µg/ml for Naftopidil. The Limit of detection (LOD) was found to be 0.0327 µg/ml and Limit of quantitation (LOQ) was found to be 0.0991 µg/ml.

2. **Linearity range:** It is the interval in which the response is directly proportional to the concentration between the upper and lower levels. It was found to be equal to 2-12 µg/ml.

3. **Target range:** It is that concentration which is 80%, 100% and 120% of the target concentration. These were equal to 5.6 µg/ml, 7.0 µg/ml and 8.4 µg/ml.

4. **Target concentration:** It is defined as the concentration, which is equal to the midpoint of linearity range. A value of target concentration for was found 7.0 µg/ml.

Accuracy
Accuracy of analytical method expresses the closeness of agreement between the value which is expected either as conventional true value or an accepted reference value and the value found. The results of analysis, obtained in three groups containing three replicate experiments
with API and different tablet dosage forms, had good agreement with the labeled amount of the drug. The results are shown in Table 4.

Table 4: Recovery study of Naftopidil in tablet dosage form.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Conc. before spiking $C_1$ (μg/ml)</th>
<th>Reference Std. added $C_2$ (μg/ml)*</th>
<th>Conc. after spiking $C_3$ (μg/ml)*</th>
<th>Percent recovery $(C_3-C_1)*100/C_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>5.6</td>
<td>7</td>
<td>12.7</td>
<td>101.42</td>
</tr>
<tr>
<td>2.</td>
<td>7</td>
<td>7</td>
<td>14.1</td>
<td>101.43</td>
</tr>
<tr>
<td>3.</td>
<td>8.4</td>
<td>7</td>
<td>15.4</td>
<td>100.00</td>
</tr>
<tr>
<td>Mean ± SD Naftopidil</td>
<td></td>
<td></td>
<td>100.95 ± 0.671</td>
<td></td>
</tr>
</tbody>
</table>

The mean recovery was found to be 100.95 % for Naftopidil. The limit for mean % recovery is 100-101.43% and both values were within the limit, hence it can be said that the proposed method was accurate.

**Precision**

**Repeatability**

Repeatability precision was determined by using six time repetitions of the single target concentration that is equivalent 100% level of target range that is 7μg/ml for Naftopidil. The results are shown in Table 5.

Table 5: Data showing repeatability analysis

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameter</th>
<th>Naftopidil</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Mean ± SD</td>
<td>108901 ± 886.45</td>
</tr>
<tr>
<td>2.</td>
<td>% RSD</td>
<td>0.8139</td>
</tr>
</tbody>
</table>

The repeatability study which was conducted on the solution having the concentration of about 7μg/ml Naftopidil (n =6) showed RSD of 0.8139%. Thus, it can be concluded that the analytical technique showed good repeatability.

**Intermediate precision**

**A. Intra-day precision:**

For intra-day precision studies the drug having concentration value 80%, 100% & 120% of the target concentration (n = 3), were injected in triplicate at same day at different times periods into the HPLC system. The result are shown in Table 6
Table 6: Data for the intra-day precision analysis

<table>
<thead>
<tr>
<th>Conc. (µg/ml) Naftopidil</th>
<th>Time</th>
<th>Mean Peak Area* (µV*sec)</th>
<th>Mean Conc. Found* (µg/ml)</th>
<th>Mean ± SD</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.6</td>
<td>9 AM</td>
<td>933400</td>
<td>5.61</td>
<td>5.60 ± 0.050</td>
<td>0.892</td>
</tr>
<tr>
<td></td>
<td>11 PM</td>
<td>933418</td>
<td>5.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 PM</td>
<td>933510</td>
<td>5.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>9 AM</td>
<td>104855</td>
<td>7.20</td>
<td>7.10 ± 0.132</td>
<td>1.85</td>
</tr>
<tr>
<td></td>
<td>11 PM</td>
<td>104726</td>
<td>7.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 PM</td>
<td>103492</td>
<td>6.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.4</td>
<td>9 AM</td>
<td>129710</td>
<td>8.45</td>
<td>8.44 ± 0.040</td>
<td>0.473</td>
</tr>
<tr>
<td></td>
<td>11 PM</td>
<td>129989</td>
<td>8.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 PM</td>
<td>129789</td>
<td>8.40</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean% RSD Naftopidil 1.07

Intraday study showed a RSD of 1.07 % for Naftopidil. It showed that the mean %RSD was found to be within acceptance limit (≤2%). Thus, it can be concluded that the analytical technique showed a good intra-day precision.

Inter-day precision

For inter-day studies the drug having concentration value 80%, 100% & 120% of the target concentration (n = 3), were injected in triplicate into the HPLC system at three different days. The results are shown in table 7

Table 7: Data for the inter-day precision analysis.

<table>
<thead>
<tr>
<th>Conc.µg/ml Naftopidil</th>
<th>Day</th>
<th>Mean Peak Area* (µV*sec)</th>
<th>Mean Conc. Found* (µg/ml)</th>
<th>Mean± SD</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.6</td>
<td>DAYI</td>
<td>933410</td>
<td>5.61</td>
<td>5.59 ± 0.037</td>
<td>0.661</td>
</tr>
<tr>
<td></td>
<td>DAYII</td>
<td>933415</td>
<td>5.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DAYIII</td>
<td>933520</td>
<td>5.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>DAYI</td>
<td>104595</td>
<td>7.01</td>
<td>7.00 ± 0.055</td>
<td>0.782</td>
</tr>
<tr>
<td></td>
<td>DAYII</td>
<td>104856</td>
<td>7.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DAYIII</td>
<td>103682</td>
<td>6.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.4</td>
<td>DAYI</td>
<td>129930</td>
<td>8.35</td>
<td>8.40 ± 0.050</td>
<td>0.595</td>
</tr>
<tr>
<td></td>
<td>DAYII</td>
<td>129269</td>
<td>8.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DAYIII</td>
<td>129929</td>
<td>8.41</td>
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</tbody>
</table>

Mean% RSD Naftopidil 0.679

Inter day study showed a RSD of 0.679 % for Naftopidil. It showed that the mean %RSD was found to be within acceptance limit (≤2%). Thus, it can be concluded that the analytical technique showed a good inter day precision.
Limit of detection and Limit of quantification

Limit of detection is the lowest amount of analyte that can be detected but not quantitated as an exact value and Limit of quantitation is the lowest amount of analyte that can be quantitatively determined in a sample with suitable precision and accuracy.

The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

LOD = 3.3(SD/S) and

LOQ = 10(SD/S)

Where, SD = Standard deviation of the response
S = Slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte. The results are shown in Table 8.

Table 8: Limit of detection and quantitation

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Slope</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16414</td>
<td>-22950</td>
</tr>
<tr>
<td>2</td>
<td>16230</td>
<td>-22652</td>
</tr>
<tr>
<td>3</td>
<td>16348</td>
<td>-22568</td>
</tr>
<tr>
<td>4</td>
<td>16411</td>
<td>-22495</td>
</tr>
<tr>
<td>5</td>
<td>16325</td>
<td>-22659</td>
</tr>
<tr>
<td>6</td>
<td>16359</td>
<td>-22783</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mean ± SD</th>
<th>LOD</th>
<th>LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>16347± 162.09</td>
<td>0.0327 μg/ml</td>
<td>0.0991 μg/ml</td>
</tr>
</tbody>
</table>

The LOD was found to be 0.0327μg/ml and LOQ was found to be 0.0991μg/ml which showed that sensitivity of the method was high.

ASSAY

Twenty tablets (Nafodil 75) were accurately weighed. These tablets were finely powdered and triturated well. The powder sample equivalent to 75 mg of Naftopidil was weighed and transferred to a 100 ml volumetric flask and 10 ml of methanol was added in it and sonicated for 15 minute to dissolve. The volume was made up to the mark with mobile phase (methanol: water 80:20) and taken 1 ml of this solution and further diluted up to 10 ml with mobile phaseto obtain concentration of 75μg/ml for Naftopidil. This solution was filtered through 0.45 μm membrane filter and sonicated to degas. The prepared solution was injected in five replicates into the HPLC system and the observations were recorded. These solutions were analyzed and percent recovery of Nafodil 75 tablet was determined.
Table 9: Estimation of Naftopidil

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Label claim (mg)</th>
<th>Conc. Mean ± SD</th>
<th>Mean % ± SD</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nafodil 75</td>
<td>75 mg Naftopidil</td>
<td>74.8625 ± 0.1537</td>
<td>99.81 ± 0.384</td>
<td>0.3847</td>
</tr>
</tbody>
</table>

The tablet content was found to be 74.86 mg/cap (99.81%) for Naftopidil in Nafodil 75 tablets.

SUMMARY & CONCLUSION

Table 10: Summary Table

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Specificity</td>
<td>No interference was found with respect to excipients</td>
</tr>
<tr>
<td>2.</td>
<td>Linearity (Correlation coefficient r)</td>
<td>0.999</td>
</tr>
<tr>
<td>3.</td>
<td>Range</td>
<td>2-12 PPM</td>
</tr>
<tr>
<td>4.</td>
<td>Accuracy (% Recovery)</td>
<td>Intra-day (n=3) = 1.070</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inter-day (days=3) = 0.679</td>
</tr>
<tr>
<td>5.</td>
<td>Precision % RSD</td>
<td>Intra-day (n=3) = 1.070</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inter-day (days=3) = 0.679</td>
</tr>
<tr>
<td>6.</td>
<td>LOD</td>
<td>0.0327</td>
</tr>
<tr>
<td>7.</td>
<td>LOQ</td>
<td>0.0991</td>
</tr>
</tbody>
</table>

CONCLUSION

A simple accurate, precise method was developed for the estimation of the Naftopidil in Tablet dosage form using RP HPLC. Retention time of Naftopidil was found to be 2.4 min. %RSD of the Naftopidil for intraday precision and inter day precision were found to be 1.070 and 0.679 respectively. %Recover was Obtained as 100.95% for Naftopidil. LOD and LOQ values were obtained from regression equations of Naftopidil was 0.0327 µg/ml and 0.0991 µg/ml respectively. Regression equation of Naftopidil was y = 16414.x ± 2295.5. Correlation coefficient for Naftopidil was found to be 0.999. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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