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Ultra Performance Liquid Chromatography Method for the Determination of Degradation Impurities of Naftopidil in Naftopidil Orally Dispersible Tablets

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ABSTRACT

This paper describes a strategy for the systematic development and validation of stability-indicating method of the determination of degradation impurities present in Naftopidil Orally dispersible tablets. Efficient separation is achieved in 75mm length x 2.1mm ID, Octadecyl column with 3µ particle size. Using pH 3.2 phosphate buffer and acetonitrile as mobile phase in gradient pump mode. Flow rate was selected 0.4mL.min^{-1}with a detection wavelength of 210nm. Validation parameters such as specificity, linearity, precision, accuracy, determination of LOD, LOQ and robustness were evaluated as per ICH guidelines. The validated Reverse phase –Ultra Performance liquid chromatography (RP-UPLC) method was successfully applied to the quantitative determination of impurities of Naftopidil in Naftopidil Orally Dispersible tablet dosage forms, helping to improve quality control and to assure therapeutic efficacy at reduced run time of minutes.

Keywords: Naftopidil, Forced Degradation, RP-UPLC and Stability Indicating

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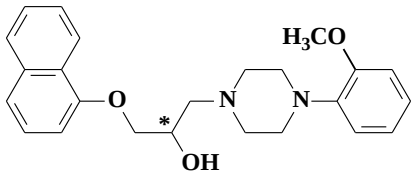
INTRODUCTION

Naftopidil is for the treatment of lower urinary tract symptoms compatible with benign prostatic hyperplasia. Benign prostatic hyperplasia (BPH) is a common condition in aging men causing lower urinary tract symptoms (LUTS). Treatment aims are to relieve symptoms and prevent disease progression. Of the different alpha-1 adrenergic receptors (ARs) in the prostate, alpha-1a receptors are known to be central to prostatic smooth-muscle contraction[1-2]. Recent studies have shown that patients with BPH may also have a predominance of alpha-1d receptors. Naftopidil is chemically described as 1-[4-(2-methoxyphenyl) piperazin-1-yl]-3-(1-naphthyloxy) propan-2-ol. An extensive literature survey revealed that few bio analytical LC-MS/MS methods are used for the determination of Naftopidil in human plasma[3]. The reported HPLC methods[4-9] were not capable to separate the peaks of impurities from Naftopidil. The literature survey also revealed that there was no stability-indicating RP-UPLC method for the determination of process and degradation-related impurities formed under the stress conditions in Naftopidil. Efficiency is the primary separation parameter behind Ultra performance liquid chromatography (UPLC) since it relies on the same selectivity and retentivity as HPLC. Smaller particles provide not only increased efficiency, but also the ability to work at increased linear velocity without a loss of efficiency, providing both resolution and speed. Instrument technology also had improved truly taking advantage of the increased speed, superior resolution and sensitivity afforded by smaller particles. UPLC System has been designed for low system and dwell volume to minimize dispersion and take full advantage of small particle technology. Keeping in view of both sophisticated instrumental and column technology, a shorter version of method was developed for Naftopidil Orally Dispersible tablets for determination of organic impurities.

In this paper we described about development and validation of related substances method for precise and accurate quantification of six potential impurities (process or degradation) in Naftopidil Orally Dispersible tablets as per International Conference on Harmonization (ICH) recommendation. Forced degradation study was conducted to determine product breakdown levels and preliminary degradation kinetics, and to identify degradant species. Forced degradation or stress testing studies are part of the development strategy and are also an integral component of validating analytical method that indicate stability and detect impurities. This relates to the specificity section of the validation studies as recommended by ICH. Gaining insight into degradation pathways, and discernment of degradation products in formulations that are related to drug substances versus those that are related to other ingredients of a formulation.

Reliable chemical stability testing data can show how a drug product changes over time with influence of environmental factors. To identify these factors analytical method should be stability-indicating and fully validated as per USP and ICH guideline recommendation[10-18].



** asymmetric centre*

Figure 1: Naftopidil chemical structure

MATERIALS AND METHODS

Instruments:

The LC system, used for method development and method validation was Waters- Acquity UPLC equipped with separation module consisting of Binary gradient pump, Auto Sampler, thermostated column compartment, Acquity UPLC Photo diode array detector, Auto sampler thermostated, Computer with windows based Empower 3 Method validation manager software. The output signal was monitored and processed using Empower 3 software. Column used for chromatography was ACE-3, C18 (75 mm x 2.1 mm id) with 3.0 µm particle size.

Chemicals:

Naftopidil drug substance, impurities of Naftopidil, Naftopidil Orally Dispersible tablets (Brand Name: Orient, china) generously sponsored by Aurobindo pharma limited. Acetonitrile (UPLC grade from Merck chemicals) and methanol (UPLC grade from Merck chemicals). Ultrapure water is prepared by using Millipore Milli-Q plus water purification system. All chemicals and reagents were used as such without further purification.

The possible impurities that may be present in Naftopidil are Dihydroxypiperazine derivative [DPD] (Impurity-1), 3-(1-Naphthyloxy)-1-Piperazinylpropan-2-ol (Impurity-2), Diol (Impurity-3), C4 Alkyl Naftopidil (Impurity-4), Desmethyl Naftopidil (Impurity-5) and Alpha Naphthol (Impurity-6). The chemical structures have been illustrated in Figure 2,3,4,5,6 and 7.

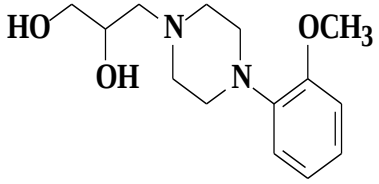


Figure 2: Chemical structure of Dihydroxypiperazine Derivative: 3-[4-(2-Methoxyphenyl) Piperazin-1-Yl] Propane-1,2-Diol (DPD) (Impurity-1)

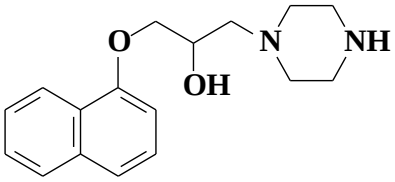


Figure 3: Chemical structure of 3-(1-Naphthyloxy)-1-Piperazinylpropan-2-ol (Impurity-2)

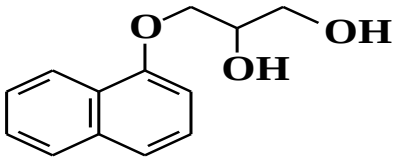


Figure 4: Chemical structure of Naftopidil diol (Impurity-3)

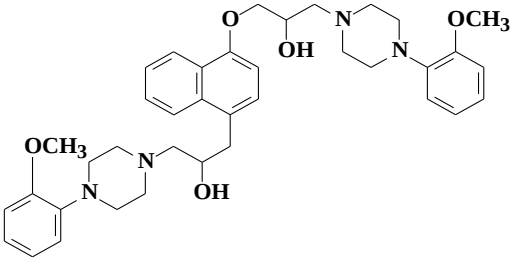


Figure 5: Chemical structure of C4-alkyl Naftopidil: 1-(4-(2-hydroxy-3-[4-(2-methoxy phenyl) piperazinyl] propyl) naphthyloxy)-3-[4-(2-methoxyphenyl) piperazinyl] propan-2-ol (Impurity-4)

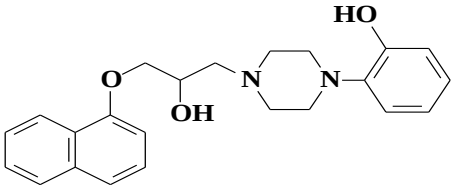


Figure 6: Chemical structure of Desmethyl Naftopidil: 4-(2-Hydroxyphenyl)-A-[(1-Naphthalenyloxy) Methyl-1-Piperazineethanol (Impurity-5)

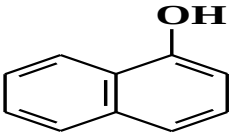


Figure 7: Chemical structure of α-Naphthol (or) 1-Naphthol (Impurity-6)

Method development and optimization for an UPLC method:

Aim of this study was to develop the chromatographic system which was capable to separate Naftopidil from its process and degradation impurities with reduced run time. pKa of Naftopidil was found to be at about 7.3 and it was in basic nature. Drug product was not official or cited in any pharmacopoeia and there was a limited analytical literature was available on this drug

product. Since Naftopidil was in basic nature, it was proposed to keep the mobile phase pH on acidic side to reduce the silanol effect from the column. Trails were initiated using 20 mM (milli molar) monobasic phosphate buffer at different acidic pH (range pH 2.5 to 3.5) as mobile phase-A and Acetonitrile as mobile phase-B. Considering nature of impurities present in drug compound, it is preferred to choose more aqueous buffer for initial elution purpose. Initially different gradient programs at a flow rate of 0.4 mL per minutes were proposed for optimum separation of all the impurities. Different column chemistries were tried during initial trail purpose. However ACE-3, C18 (75 mm x 2.1 mm id) with 3.0 µm particle size column has shown better specificity. The same column was used for entire method development work.

To know the elution pattern of these impurities, solutions of impurity mixture were prepared at 0.2 % of the sample concentration for Impurity-1, Impurity-2, Impurity-3, Impurity-5 and Impurity-6 (0.6µg/mL) and 0.5% for Impurity-4 (1.5µg/mL) respectively. Spectral data for individual impurities has shown wavelength maxima at about 210nm, and hence the same wavelength has chosen for quantification purpose.

The percentage of organic ratio in mobile phase B was played a key role in separation of all process and degradation impurities which may arise due to Naftopidil. After several logical trials with different flow rates, change in pH of buffer in Mobile phase A and organic ratio in mobile phase B, chromatographic condition was established with optimal resolution between all known impurities of greater than 1.5

Finalized chromatographic conditions

Finalized column for chromatography was ACE-3, C18 (75 mm x 2.1 mm id) with 3.0 µm particle size.

Table 1. Gradient programme

Time in minutes	Flow in mL	Mobile Phase A (%)	Mobile Phase B (%)
0.0	0.4	98	2
3.0	0.4	98	2
10.0	0.4	73	27
10.1	0.4	98	2
13.0	0.4	98	2

The mobile phase- A containing 20 mM of monobasic potassium phosphate in water (2.72g of monobasic potassium phosphate in 1000 mL of water), adjusted the pH to 3.2 ± 0.05 with dilute ortho phosphoric acid. Filtered through 0.22µ membrane filter. Acetonitrile was used as mobile phase-B. The flow rate of the mobile phase was 0.4 mL.min–1 with gradient elution mode (Table 1). The column temperature was maintained at 40°C and the detection wavelength was fixed at

210 nm. The injection volume was 2µL. Diluent consists of 30% Acetonitrile in water.

In the finalized chromatographic conditions, typical retention times of Impurity 1, Impurity 2, Impurity 3, Impurity 4, Impurity 5 and Impurity 6 are 0.74, 2.21, 5.56, 5.98, 6.78 and 7.3 respectively. For general system suitability data refer Table 5.

Table 5. General system suitability data

Name of the component	USP Resolution	USP Tailing factor	USP Theoretical plates
Impurity 1	--	1.05	250
Impurity 2	6.8	1.41	1205
Impurity 3	11.7	1.33	5873
Impurity 4	1.59	1.47	9875
Impurity 5	3.23	1.39	11713
Impurity 6	1.71	1.28	8347
Naftopidil	2.13	1.68	7460

PREPARATION OF SOLUTIONS

Preparation of standard solution

Initial Standard stock solution of Naftopidil (0.38 mg/mL) was prepared by dissolving in 10 mL of acetonitrile followed by make up to the volume with diluent. This stock solution was further diluted to obtain a concentration of 0.60µg/mL. All impurities were prepared by initially dissolving in an appropriate amount of acetonitrile.

Preparation of sample solution

Finely powder not less than 10 tablets in a suitable pestle & mortar. Weigh and transfer accurately a quantity of tablet powder equivalent to about 75 mg of Naftopidil into a 250 mL clean, dry volumetric flask, add about 25 mL of acetonitrile and disperse, add about 140ml of diluent sonicate at room temperature for about 20 minutes with intermittent shaking. Make up to the volume with diluent and mix. Filter through suitable 0.45 µ membrane filter. Final Concentration of sample solution is 0.3mg/mL (300 µg/mL).

Chromatographic System suitability parameters

The column efficiency as determined from the Naftopidil peak is not less than 5000 USP plate count and the Symmetry factor for the same peak is not more than 2.0. RSD for peak areas of six injections of the standard solution is not more than 5.0%. For data refer Table 6.

Table 6 Chromatographic system suitability data

Name of the component	USP Theoretical plates	USP Tailing factor	% RSD for 6 replicate injections
Naftopidil	17695	1.29	0.9

ANALYTICAL METHOD VALIDATION

Naftopidil Dispersible tablets are available in different strengths such as 25mg, 50mg and 75mg per tablets. However 75mg strength was considered for entire validation experimentation. The developed method was validated for Specificity, Forced degradation studies, Precision, Sensitivity (Limit of detection and Limit of Quantification), Linearity, Range, Accuracy and Robustness as per ICH recommendation.

Specificity and Stress studies

The specificity of the developed method for Naftopidil Dispersible tablets is determined in the presence of Impurity 1, Impurity 2, Impurity 3, Impurity 5 and Impurity 6 at a concentration of 0.6µg/mL, and 1.5µg/mL for Impurity 4 and its corresponding degradation products. Concentrations of impurities were fixed based on nature of the impurities. The stress conditions used for degradation study are Acid hydrolysis (2M HCl / 25°C / 60 min), Base hydrolysis (2M NaOH / 25°C / 60 min), Oxidation (30% H2O2 / 25°C / 30 min), Thermal (80°C /5 hours), Humidity (70%RH / 5 Hours) and Photolytic (white fluorescent 1.2 million lux hours UV 200 watt hr/m^2 for 7 days).

Precision

The precision of the method was checked by injecting six individual preparations of Naftopidil Orally Dispersible tablets spiked with 0.2% level for Impurity 1, Impurity 2, Impurity 3, Impurity 5 and Impurity 6 and 0.5% level for Impurity 4. The percentage RSD for % w/w of each impurity is calculated. The intermediate precision (Ruggedness) of the method was evaluated by different analyst using different column and different UPLC instrument on different day.

Sensitivity (Limit of detection and Limit of Quantification)

For the establishment of LOD and LOQ levels, a series of test solutions were prepared from 1 to 150% with respective impurity specification level by diluting the impurity stock solution to the required concentration. Linearity curves were drawn by plotting concentration versus area of the individual impurity. From these plots, LOD and LOQ were predicted from the formulae $3.3\sigma/S$ and $10\sigma/S$ respectively where σ is the standard deviation of the response and S is the slope of the linearity curve. Precision was performed at predicted LOD and LOQ values and finalized the LOD and LOQ levels.

Linearity and Range

Linearity curves were plotted from the finalized LOQ level to 150% of the impurity specification level. The correlation coefficient, slope and Y-intercept of the Linearity curve are calculated for

each impurity.

Accuracy

A known amount of the impurity stock solutions were spiked to the samples at 50%, 100% and 150% of the analyte concentration. The % w/w of recoveries for all the impurities was calculated. Each concentration level is prepared for triplicate preparation.

Solution Stability:

In order to demonstrate the stability of both reference and sample solutions, these solutions were injected immediately after preparation and at periodical intervals by maintaining at room temperature.

Robustness

To determine the robustness of the developed method, experimental conditions are deliberately changed and the impact of the variation on each impurity was evaluated. The flow rate of the mobile phase is 0.4 mL/min. To study the effect of flow rate ± 0.1 unit was changed i.e., 0.3 and 0.5 mL/min. The effect of column temperature (actual 40°C) is studied at 35°C and 45°C. The effect of pH of mobile phase (actual pH is 3.2) is studied at pH 3.0 and pH 3.4 respectively. For gradient programme variation, the composition of mobile phase-B was changed ± 2 absolute. For wavelength variation, ± 5 nm was changed from the working wavelength i.e., 210nm.

RESULTS AND DISCUSSION

All degradations conditions were performed using finalized chromatographic conditions.

Specificity and Stress studies

Stress studies on Naftopidil Orally Dispersible tablets under different stress conditions suggested the following degradation behavior.

Degradation in Acid stress condition

There is no significant degradation for Naftopidil.

Degradation in Base stress condition

There is no significant degradation for Naftopidil. However one unknown is generated at about RRT 1.24.

Degradation in Peroxide stress condition

There is a significant degradation is observed for Naftopidil. Unknowns are generated at about RRT 0.35, RRT 0.74 and RRT 0.95.

Degradation in Thermal stress condition

There is no significant degradation is observed for Naftopidil.

Degradation in Humidity stress condition

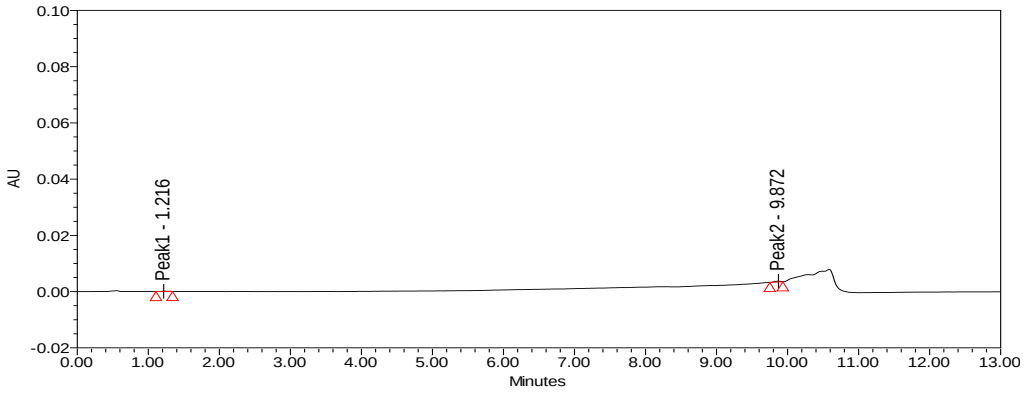
There is no significant degradation is observed for Naftopidil.

Degradation in photolytic stress condition

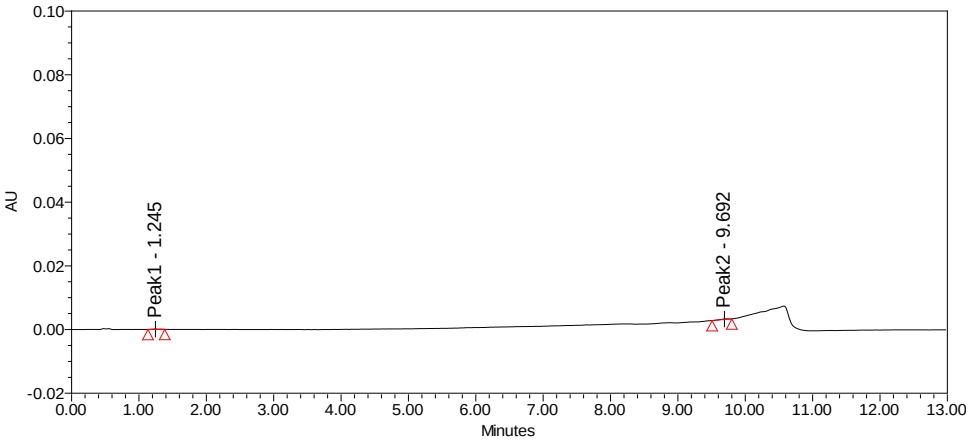
There is no significant degradation is observed for Naftopidil. (Figure 8, Table 2)

Table 2. Summary of forced degradation study

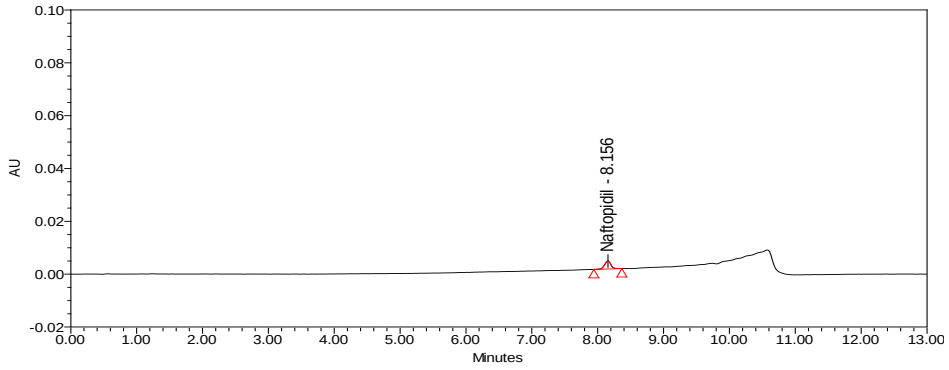
Stress condition	Time	% impurities+ %Degradation products Naftopidil	Major Appeared impurities
Acid hydrolysis (2M HCl / 25°C)	60 minutes	0.06	Impurity 4
Base hydrolysis (2M NaOH / 25°C)	60 minutes	0.28	Unknown @ RRT 1.24
Oxidation stress sample (30% H2O2 / 25°C)	30 minutes	7.96	Unknown @ RRT 0.35
Thermal stress sample(80°C /5 hours)	5 hours	0.05	Impurity 4
Humidity stress sample(70%RH / 25°C)	5 hours	0.04	Impurity 4
Photolytic stress sample(White Flouoscent Light 1.2 Millon Lux Hrs Uv 200 Watts/M^2)	7 Days	0.05	Impurity 4 and Impurity 5



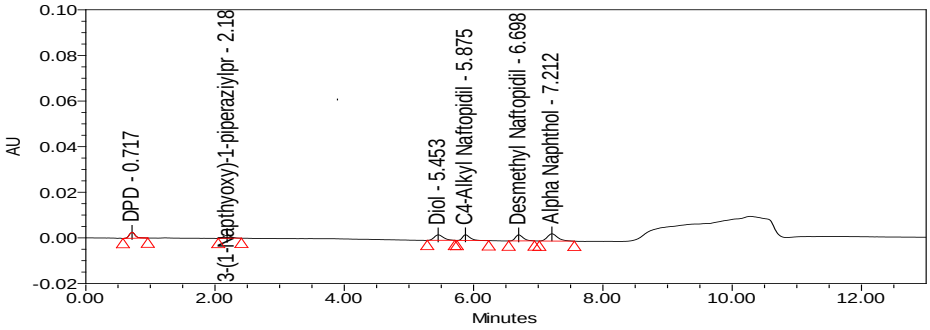
a)Diluent



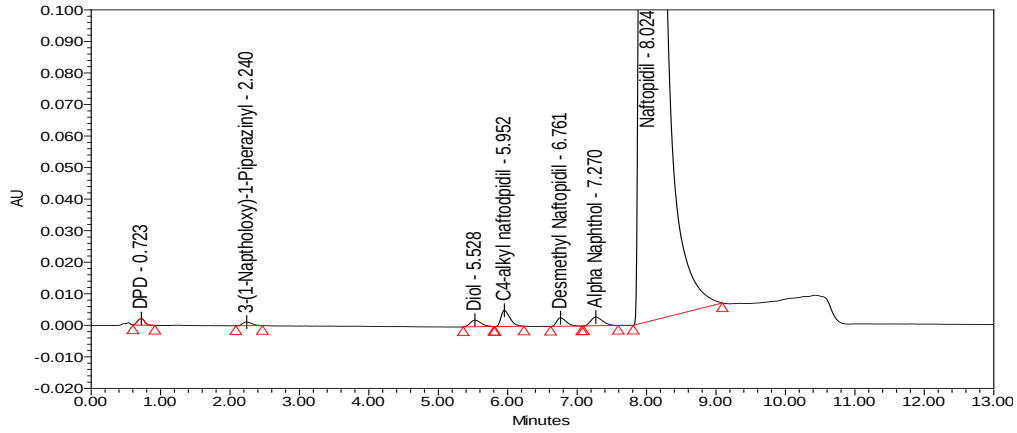
b)Placebo



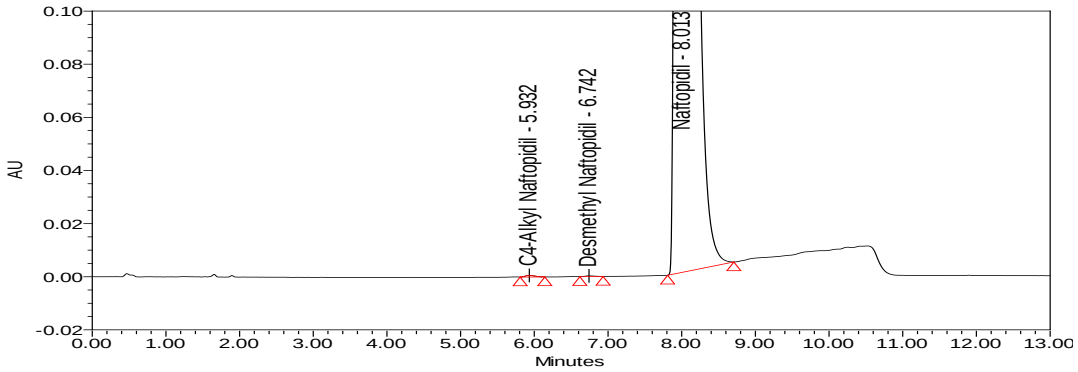
c) Diluted Standard Solution



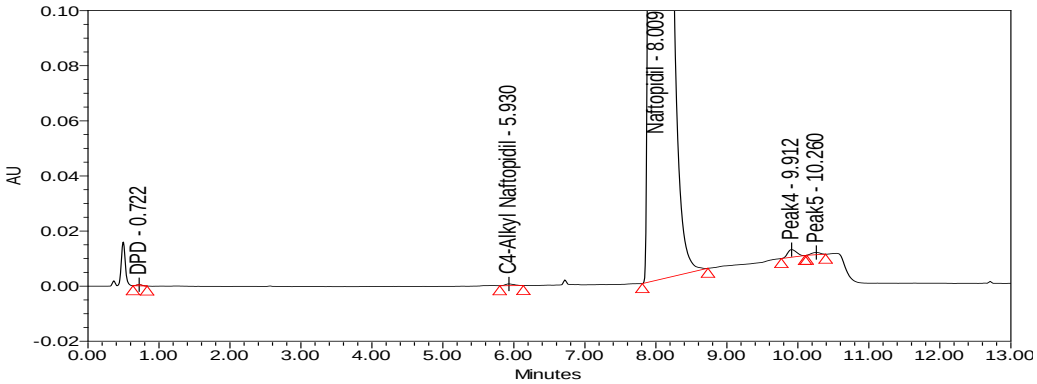
d)All impurity mixture at specification level



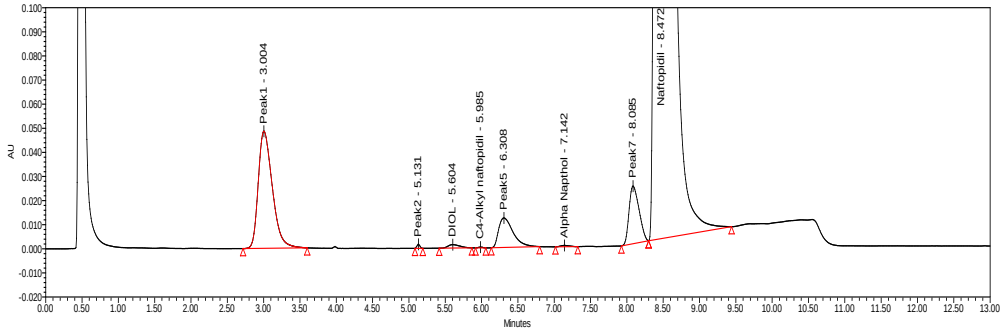
e) Sample Spiked with Impurities



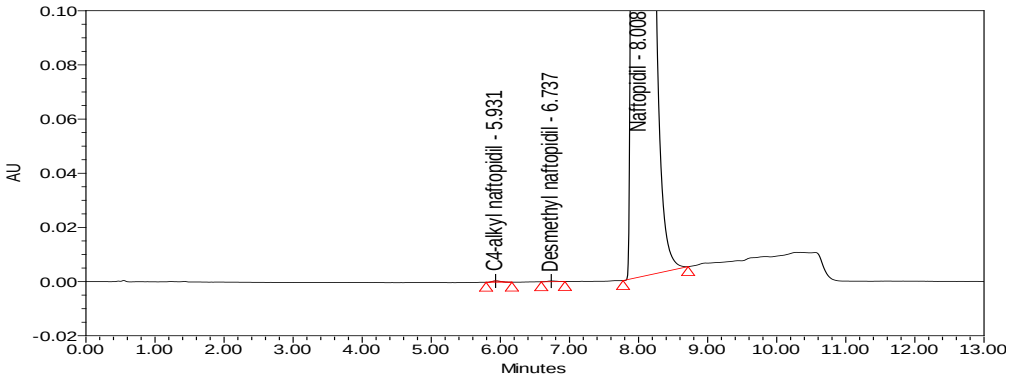
f) Acid Stress Sample



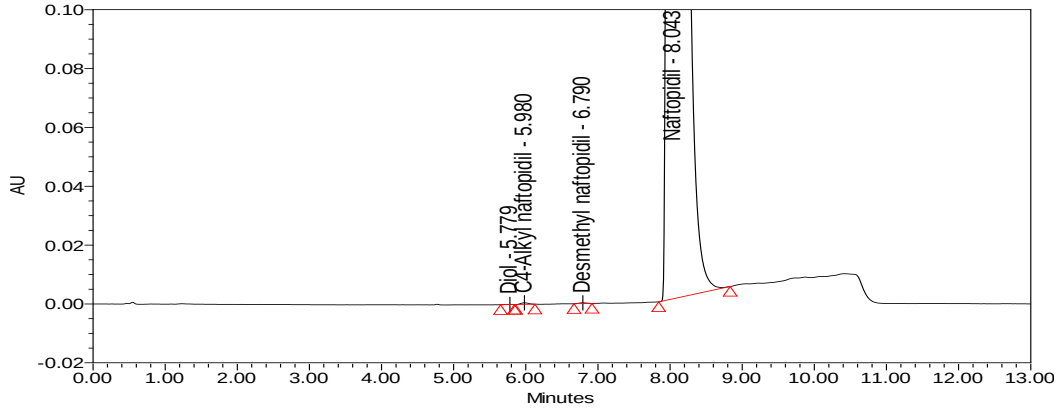
g) Base Stress Sample



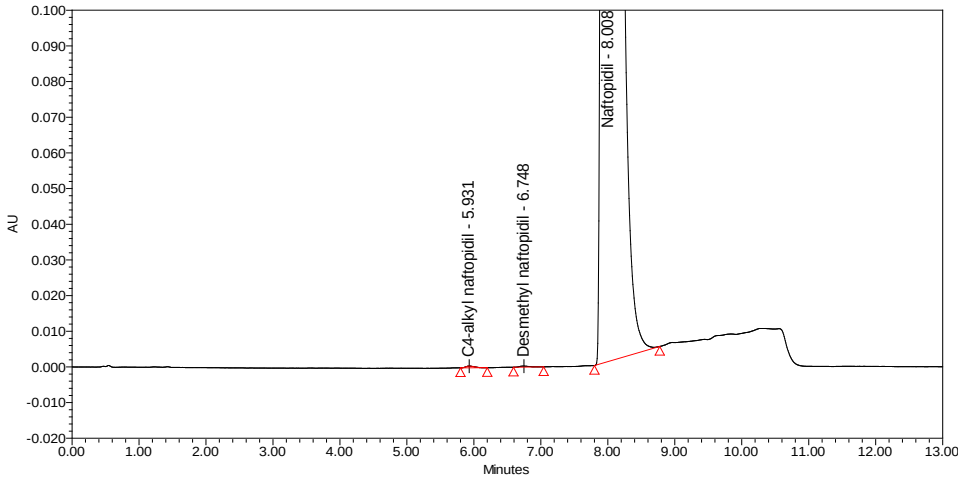
h) Oxidation Stress Sample



i) Thermal Stress Sample



j) Humidity Stress Sample



k)Photolytic stress sample

Figure 8: Typical chromatogram of Diluent, placebo, Diluted Standard solution, All impurity mixture at specification level, Naftopidil spiked with impurities & Stress sample chromatograms.

METHOD VALIDATION

Precision

The percentage RSD of %w/w of Impurity 1, Impurity 2, Impurity 3, Impurity 4, Impurity 5 and Impurity 6 is 2.5, 2.5, 2.4, 2.8, 2.4, and 3.2 respectively confirming the good precision of the developed method. The % RSD obtained in intermediate precision study for Impurity 1, Impurity 2, Impurity 3, Impurity 4, Impurity 5 and Impurity 6 is 1.3, 3.4, 2.3, 1.4, 0.5 and 1.8 respectively confirming the intermediate precision.

Sensitivity (Limit of detection and Limit of Quantification)

The Limit of Detection for Impurity 1, Impurity 2, Impurity 3, Impurity 4, Impurity 5 and Impurity 6 is 0.018%, 0.018%, 0.022%, 0.055%, 0.019% and 0.017% respectively (of analyte concentration, i.e. 300 µg/mL). The Limit of Quantification for Impurity 1, Impurity 2, Impurity 3, Impurity 4, Impurity 5 and Impurity 6 is 0.045%, 0.045%, 0.054%, 0.110%, 0.048% and 0.043% respectively (of analyte concentration, i.e. 300 µg/mL for Naftopidil).

Linearity and Range

Calibration curve obtained by the least square regression analysis between peak area and concentration showed linear relationship with a correlation coefficient of greater than 0.995 over the calibration ranges tested. Linear calibration plot for the related substances method is obtained over the calibration range LOQ to 150%. The results show an excellent correlation obtained between peak area and concentration of Naftopidil and all the impurities. (Table 3)

Table 3. Linearity table

Name of the component	Trend line equation	Range	Correlation coefficient	Intercept	Residual sum of squares
Impurity 1	y = 30247x + 185	0.003-0.872	0.9992	185	403
Impurity 2	y = 20272x - 125	0.001-0.812	0.99848	-125	348
Impurity 3	y = 36363x - 264	0.0082-0.977	0.99982	-264	251
Impurity 4	y = 17495x - 1360	0.001-2.318	0.99587	-1360	1397
Impurity 5	y = 39795x - 468	0.0032-0.870	0.99975	-0.468	299
Impurity 6	y = 69918x -375	0.0062-0.872	0.99987	-375	366
Naftopidil	y = 42918x - 376	0.0062-0.912	0.99965	-376	392

Accuracy

Accuracy was assessed from three replicate determinations of three different levels including 50%, 100% and 150% of the specification level of the impurities. The observed recovery results were found in the range between 90 to 110% with the RSDs lower than 5.0% demonstrating that the method is accurate within the desired range. (**Table 4a, Table 4b and Table 4c**).

Table 4a. Table for Accuracy study for Naftopidil impurity 1 and impurity 2

Sample spiked at level	Impurity 1		% Recovery	Impurity 2		% Recovery
	Amount added (%w/w)	Amount recovered (%w/w)		Amount added (% w/w)	Amount recovered (%w/w)	
50% sample -1	0.083	0.082	98.8	0.1	0.096	96
50% sample -2	0.081	0.081	100	0.098	0.094	95.9
50% sample -3	0.08	0.08	100	0.096	0.094	97.9
100% sample -1	0.158	0.155	98.1	0.19	0.177	93.2
100% sample -2	0.162	0.159	98.1	0.195	0.182	93.3
100% sample -3	0.159	0.156	98.1	0.192	0.181	94.3
150% sample -1	0.258	0.253	98.1	0.292	0.293	100.3
150% sample -2	0.254	0.247	97.2	0.287	0.294	102.4
150% sample -3	0.246	0.24	97.6	0.278	0.283	101.8

Table 4b. Table for Accuracy study for Naftopidil impurity 3 and impurity 4

Sample spiked at level	Impurity 3		% Recovery	Impurity 4		% Recovery
	Amount added (%w/w)	Amount recovered (%w/w)		Amount added (% w/w)	Amount recovered (%w/w)	
50% sample -1	0.108	0.105	97.2	0.387	0.386	99.7
50% sample -2	0.106	0.102	96.2	0.381	0.368	96.6
50% sample -3	0.104	0.103	99.0	0.374	0.370	98.9
100% sample -1	0.206	0.200	97.1	0.74	0.744	100.5
100% sample -2	0.211	0.207	98.1	0.757	0.761	100.5
100% sample -3	0.207	0.206	99.5	0.746	0.752	100.8
150% sample -1	0.294	0.294	100.0	1.322	1.389	105.1
150% sample -2	0.289	0.281	97.2	1.299	1.370	105.5
150% sample -3	0.280	0.278	99.3	1.259	1.325	105.2

Table 4c. Table for Accuracy study for Naftopidil impurity 5 and impurity 6

Sample spiked at level	Impurity 5 Amount added (%w/w)	Amount recovered (%w/w)	% Recovery	Impurity 6 Amount added (% w/w)	Amount recovered (%w/w)	% Recovery
50% sample -1	0.105	0.098	93.3	0.085	0.081	95.3
50% sample -2	0.104	0.095	91.3	0.083	0.080	96.4
50% sample -3	0.102	0.092	90.2	0.082	0.079	96.3
100% sample -1	0.202	0.187	92.6	0.162	0.153	94.4
100% sample -2	0.206	0.194	94.2	0.166	0.155	93.4
100% sample -3	0.203	0.188	92.6	0.163	0.151	92.6
150% sample -1	0.309	0.291	94.2	0.281	0.273	97.2
150% sample -2	0.303	0.282	93.1	0.277	0.264	95.3
150% sample -3	0.294	0.275	93.5	0.268	0.258	96.3

Solution Stability:

No significant changes are observed in the area of Impurity 1, Impurity 2, Impurity 3, Impurity 4, Impurity 5 and Impurity 6 during solution stability experiment. The solution stability experiment data confirms that standard and sample solutions were stable up to the study period of 28 hours.

Robustness

Close observation of analysis results for deliberately changed chromatographic conditions Flow rate, column temperature, change in pH, wave length and change of organic component in gradient programme revealed that there is no significant change observed in the relative retention times of the main analyte and their corresponding impurities illustrating the robustness of the method.

CONCLUSION

The proposed UPLC method enables the separation and simultaneous quantitative determination of specified and unspecified impurities of Naftopidil in Orally Dispersible tablets. The developed method is validated as per ICH requirements. The stress studies indicated that method is selective and stability indicating. UV detection at 210nm was found to be suitable without any interference from excipients. All the calibration curves obtained were found to linear with values of correlation coefficients greater than 0.995. Recovery tests confirmed the accuracy of the method. The proposed UPLC method is fast, precise, accurate, sensitive and efficient.

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