"*Development and Validation of HPLC Method for the Quantitative Estimation of Naftopidil in Bulk and Pharmaceutical Formulation*."

Thesis submitted for the Degree of Master of Pharmacy
By

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CERTIFICATE OF APPROVAL

This is to certify that thesis entitled **"*Development and Validation of HPLC Method for the Quantitative Estimation of Naftopidil in Bulk and Pharmaceutical Formulation*"** has been carried out by Mr. Avijit Das , Jadavpur University, under my supervision in the Synthetic and Natural Product Research Laboratory, Division of Medicinal and Pharmaceutical Chemistry, Department of Pharmaceutical Technology, Jadavpur University, for partial fulfillment of the requirement for completion of the Master degree in Pharmacy.

He has carried out this research work independently with great care, sincerity and attention to my entire satisfaction.

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Declaration

I hereby declare that the dissertation entitled ***"Development and Validation of HPLC Method for the Quantitative Estimation of Naftopidil in Bulk and Pharmaceutical Formulation"*** *submitted by me for the degree of Master of Pharmacy of the Jadavpur University,Kolkata - 32 is a result of my original and independent work in Jadavpur University under the guidance of Prof.(Dr.) Tapan Kumar Maity, Dept. of Pharmaceutical Technology, Jadavpur University, Kolkata – 32.*

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Acknowledgement

Completing task is never one man's effort. It is often result of invaluable contribution of a number of individual in a direct or indirect manner. This suitably applied to my dissertation work.

I offer flowers of gratitude to the all mighty god who has been the source of strength throughout my life.

My most venerable thanks to my guide ***Prof.(Dr.) Tapan Kumar Maity****, Professor, Dept. of Pharmaceutical Technology, Jadavpur University for proficient guidance, administration, invaluable, advice & constant encouragement in nurturing up this project work a grand success. He helped me immensely in the way of providing me all kind of needs.*

I owe my deep respect to ***Prof. (Dr.) Biswajit Mukherjee****, Head of the Department of Pharmaceutical Technology, Jadavpur University for his necessary help and encouragement as the Head of this Department.*

I wish wholehearted thanks to my beloved parents for their constant encouragement and blessings to complete this work successfully. I am also grateful to my family member's affection.

It's my pleasure to express my profound thanks to ***Mr. C . P . B h a r e c h , D i r e c t o r*** *, C I Laboratories, 24B B L Shah Road, Kolkata - 53 for providing me breathtaking and true encouraging environment.*

I am especially thankful to, ***Mr. S.P.Bharech (Director), Mr. N.P.Bharech (Director) and Mr.O.P Bharech (Director)*** *of* ***M/s*** *C* ***I Laboratories****, for there incredible co-operation and incessant support. There boosting attitude and creative ideas were great support to the perseverance. I had put in during my project.*

In addition to my advisors, a number of others at C I Laboratories have also had to large impact on my work. Amongst the end lees list I would thank to ***Mr. Mrinmoy Ghosh****,* ***Mr. P.K.Ghosh Mr. Bikash Chandra Mondol, Mr. Tayabur Rahaman Mallick, Mr. Biplab Mukherjee, Mr. Sumit Ganguli.***

My sincere gratitude is to Prof. (Dr.)Amalesh Samanta, Jadavpur University for his constant encouragement.

I am thankful to my seniors and juniors, ***Tanushree Singha, Tanmoy Guria , Benu Pani Sahu, S. Ramasamy, Puspita Roy, Jayatri Naskar and Dyut kumar Sarkar*** *for their valuable support and co-operation.*

I am also thankful to Mr. Biren Karan Sir for his valuable suggestion.

I wish wholehearted thanks to my beloved sister and her family for their encouragement to complete this work successfully

Date: ……

Place: Jadavpur, Kolkata *(Avijit Das)*

Certificate for Authorization

TO WHOM IT MAY CONCERN

This to certified that Mr.Avijit Das, S/o. Mr.Durgapada Das residing at village Radhaballavpur, P.O- Haripur, Dist-Hooghly Pin.-712701 is an employee at our organization pursuing M.pharm from Jadavpur University. I authorized him for doing his necessary analytical work at our laboratory. I also wish him all success in his life.

Date: For C.I Laboratories

Place:

Dedicated to My Parents

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LIST OF ABBREVIATIONS

ABBREVIATION	DISCRIPTION
Abs.	Absorbance
API	Active Pharmaceutical Ingredients
BPH	Benign Prostate Hyperplasia
cm	Centimeter
DMSO	Dimethyl Sulphoxide
HPLC	High Performance Liquid Chromatography
ICH	International Conference of Harmonization
I.P.	Indian Pharmacopoeia
LOD	Limit of Detection
LOQ	Limit of Quantification
µg	Microgram
HCl	Hydrochloric Acid
NaOH	Sodium Hydroxide
ml	Mililiter
nm	Nanometer
RS	Reference Standard
RSD	Relative Standard Deviation
RI	Reductase Inhibitor
ODS	Octa Decyl Silane

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Chapter 1
INTRODUCTION

1.0 INTRODUCTION:

Analytical methods are used for product research, product development, process control and chemical quality control purposes. Each of the techniques used in chromatographic or spectroscopic, have their own special features and deficiencies, which must be considered. Each step in the method must be investigated to determine the extent to which environment, matrix, or procedural variables can affect the estimation of analyte in the matrix from the time of collection up to the time of analysis

Pharmaceutical analysis require very precise and accurate assay methods to quantify drugs either in Pharmaceutical or biological samples. The assay methods have to be sensitive, selective, rugged and reproducible. Analytical chemistry is the qualitative and quantitative analysis of drug substances in biological fluids (mainly plasma and urine) or tissue. It plays a significant role in the evaluation and interpretation of pharmacokinetic data. The main analytical phases comprise method development, method validation and sample analysis.

1.1 Need for pharmaceutical Analysis:

- New Drug Development.
- Method Validation as for ICH Guidelines.
- Research in Pharmaceutical Sciences.
- Clinical Pharmacokinetic Studies.

1.2 Reasons for development of new methods:

- The drug or its combination is new and not official in pharmacopoeia.
- A proper analytical technique is not available in literature due to patent regulations.
- Analytical procedure is available for bulk drug but for its formulation analytical procedure is not available due to its interaction with excipients.
- Analytical procedure for drug/s in biological fluid is not available.
- The existing procedure for analysis of drug/s is lengthy, time consuming or expensive.

1.3 Modern Techniques:

The modern analytical methods of analysis can be divided in four categories:

- **Spectroscopical methods** like UV-VISIBLE spectroscopy, Infra Red-Spectroscopy (IR), Nuclear Magnetic Resonance Spectroscopy (NMR), Mass spectrometry (MS).
- **Chromatographic methods** Gas chromatography (GC), High performance liquid chromatography (HPLC), Size exclusion chromatography (SEC) High performance thin layer chromatography (HPTLC), Super critical fluid chromatography (SFC).

- **Radiometric methods** like isotopic dilution.
- **Radiometric methods** like isotopic dilution.
- **Miscellaneous methods like** pH-metry, Voltametry, Potentiometrty, Polarimetry, Nephelometry.

1.4 ICH Guideline and Method Validation:

As per ICH guide line[1] a typical analytical performance characteristic use in Method Validation are

- **Specificity (Selectivity):** is the ability to assess unequivocally the analyte in presence of components which may be expected to be present.
- **Linearity:** of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.
- **Range:** of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.
- **Accuracy:** of an analytical method is the closeness of test result obtained by that method to the true value.
- **Precision:** of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogenous sample.
- **Detection Limit**: of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated, under the stated experimental conditions.
- **Quantitation Limit:** of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.
- **Ruggedness:** of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions, such as different laboratories different analyst, different instruments, different lots of reagent, different elapsed assay times, different assay temperatures, different days, etc.
- **System Suitability:** is an integral part of many analytical procedures.

The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Some of system suitability parameters are %RSD, Tailing Factor, Resolution, Theoritical Plate.

- The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products require that stress testing is carried out to elucidate the inherent stability characteristics of the active substance. Stress testing will be carried out by the following process :-

  a) Hydrolytic degradation under acidic condition.
  b) Hydrolytic degradation under alkaline condition.
  c) Oxidative degradation.
  d) Thermal induced degradation.
  e) Photo degradation.

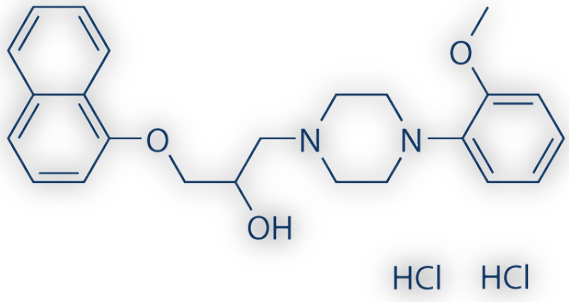
Chapter 2

DRUG PROFILE

2.0 DRUG PROFILE

2.1 *Chemical nature:*

Name:	:	Naftopidil
CAS No.:	:	571490-72
Chemical Name:	:	1-[4-(2-methoxyphenyl) piperazin-1-yl]-3-(1-naphthyloxy) propan-2-ol.
Synonyms	:	Kt611;Flivas;Avishot;Naftopil



Molecular Formula:	$C_{24}H_{28}N_2O_3$, 2HCl
Molecular Weight:	465.4126
Appearance :	White or Off -White crystalline powder.
Solubility :	Insoluble in Water, soluble in Methanol: >10 mg/mL and DMSO and Dichlormethane.
MP:	127 °C
Pka Value:	7.3
Storage temp. :	Store at room temperature.
Source:	Synthetic.

2.2 PHARMACOKINETICS:

2.2.1 Mechanism of Action:

Benign prostatic hyperplasia (BPH) is a histological condition that is commonly observed in elderly men. Benign enlargement of the prostate (BPE) due to BPH induces bladder outlet obstruction (BOO) and results in the development of lower urinary tract symptoms (LUTS). There are two mechanisms involved in the development of BOO: mechanical obstruction due to increased volume of the prostate; and functional obstruction due to increased tone of the prostatic smooth muscle.

There are two mainstays of medical treatment for LUTS suggestive of BPH (LUTS/BPH): 5α reductase inhibitors (5αRI); and α_1-adrenergic receptor (AR) antagonists (α_1 blockers).

5ARI (finasteride, dutasteride) improve BOO through reduction of the prostate volume and contribute to gradual improvement of LUTS and long term inhibition of disease progression. On the other hand, $\alpha_1$blockers improve BOO through a decrease of tone of the prostatic smooth muscle.

Several α1blockers are clinically available, including those having nonspecific affinity for α_1AR subtypes (prazosin, terazosin, doxazosin, alfuzosin) and those having specific affinity for them (tamsulosin, naftopidil, silodosin). Although several large scale studies have demonstrated recently that combination of a 5α RI and α_1 blocker is superior to either 5α RI or α_1 blocker mono therapy in terms of improvement of subjective and objective urinary symptoms as well as the long term inhibition of BPH related events such as acute urinary retention and conversion to surgical treatment, α1blockers are still essential as first line medical treatments for patients having LUTS/BPH with problems, because symptomatic improvement can be achieved rapidly with α1blockers BOO. Naftopidil, with three times greater affinity for α_1D than for the α_1AAR subtype, is an α1blocker that has been approved for clinical use for LUTS/BPH only in Japan since 1999.

Two Japanese clinical guidelines, the Clinical Guideline for Male Lower Urinary Tract Symptoms and Clinical Guideline for Benign Prostatic Hyperplasia, recommend the use of naftopidil for male LUTS/BPH as a grade A recommendation. At present, naftopidil has the second highest use, following tamsulosin, in Japan.

However, due to the limited number of English reports on Naftopidil and doctors being unable to use it in other countries, the clinical characteristics of this drug have not been sufficiently introduced. Although the English literature on Naftopidil up to January 2009 was comprehensively summarized in a review article written by Garimella *et al*, several important studies written in Japanese were missed because only reports published in English were reviewed. In this article, the efficacy and safety of Naftopidil for LUTS/BPH are reviewed based on the English literature published up to March 2011 as well as several important Japanese reports such as the results of a randomized placebo controlled trial conducted in Japan.

Naftopidil is clinically available only in Japan. There are no data derived from white or black men living in western countries. Thus, it remains unknown if the efficacy and safety of Naftopidil in the Japanese and Asian population are applicable to others. In addition, well designed prospective large scale clinical studies having adequate statistical power to draw solid conclusions are lacking. On the other hand, the possible low incidence of sexual dysfunction caused by Naftopidil is attractive. Further comparative short term and long term studies including evaluation of sexual function are mandatory. In addition, studies in real life clinical practice are necessary to apply the results obtained by randomized controlled studies to the general[2].

In healthy adult volunteers, after once oral administration of Flivas alone with 25mg, 50mg and 100mg, the Tmax is 0.45±0.21, 0.75±0.71, 0.65±0.22 hrs respectively, and the Cmax is 39.3±10.3, 70.1±32.9, 134.8±55.8mg/m1 respectively, while the half life is 15.2±4.7, 10.3±4.1, 20.1±13.7hrs. 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 hrs, 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 hrs respectively. Peak concentration and elimination half life have no significant changes to suggest that food has little influence on Flivas'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[3].

2.3 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.

2.4 DRUG INTERACTIONS:

- Dizziness
- Fainting
- Orthostatic hypotension
- Palpitations
- Flushing
- Nausea

2.5 DOSAGE AND ADMINISTRARION:

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.

2.6 PRECAUTION:

Naftopidil is contraindicated in patients with orthostatic hypotension, salt imbalance, women and children. Do not wake up suddenly from bed during morning. It can cause fall accidents due to iatrogenic orthostatic hypotension. Wakeup, sit and stand gradually after five to ten minutes after wake up4.

Chapter 3

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

3.0 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Method:

The history of chromatography is almost a century old. A botanist and physical chemist Mikhail Tswett first used the word chromatography in 1906. Chromatography is basically Greek word meaning "color writing". The technique was initially used for separation of colored substances such as plant pigments and dyestuffs. Since its invention the technique of chromatography has undergone tremendous modifications and now days various types of chromatographic techniques have been developed which can separate almost any kind of given mixture, whether colored or colorless into its constituents and to test the purity of these constituents. High pressure/performance liquid chromatography is a versatile technique adopted by many commercial and academic institutes for separation and quantitation of various drug substances. Most of the drugs in multicomponent dosage forms can be easily analyzed by high-performance liquid chromatography (HPLC), a form of liquid chromatography to separate compounds that are dissolved in solution, because of the several advantages like rapidity, specificity, accuracy, precision and ease of automation. HPLC method eliminates tedious extraction and isolation procedures. It is superior than Gas chromatography in respect of lower temperature is required during the analysis so it can be used for analysis of thermolabile analytes.

The basic principle of separation in HPLC is, the analytes gets dissolved in the mobile phase and gets distributed in the stationary phase. According to the interaction between analytes and stationary phase, the analytes elutes from the column. The chromatographic separation of analytes depends on the fact that they have different partition or distribution coefficients between the stationary and mobile phases. The variety of stationary phases used in liquid chromatography gives rise to a variety of separation mode, which includes:

i) Adsorption chromatography
ii) Liquid- liquid partition chromatography
iii) Ion-pair partition chromatography
iv) Affinity chromatography
v) Reversed phase chromatography
vi) Exclusion chromatography
vii) Ion exchange chromatography

Adsorption chromatography, also known as normal phase chromatography, where stationary phase is polar and mobile phase is non-polar is widely used in the separation of non-polar or moderately polar organic compounds. The mobile phase generally used is hexane or dichloromethane. 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 time because of their higher affinity with the stationary phase. Therefore, they take more time to elute. The majority of pharmaceutical substances are polar in nature so normal phase chromatography is not an acceptable tool for analysis of pharmaceutical substances as analysis time is longer.

In ***liquid-liquid chromatography*** support is coated with a layer of a polymer such as polyethylene glycol or a liquid, which is immiscible with the mobile phase. The relative solubility of the solutes in the mobile and stationary phases provides effective separations.

Ion pair chromatography is an alternative procedure to ion exchange chromatography. It can be further divided in to Norma phase ion pair chromatography, Reverse phase ion pair chromatography, and Soap chromatography. In normal phase, silica is used as the support and is coated with an aqueous stationary phase containing an acid (if bases are to be separated) or a base such as tetraethyl ammonium hydroxide (if acids are to be separated). An organic mobile phase such as butanol/chloroform is used. In reverse phase, a chemically bonded silica support is used along with an aqueous phase containing the required acidic or basic counter ions. Soap chromatography is a form of ion pair chromatography in which the counter ion of the ionisable compounds to be separated is a detergent for example, cetrimide and lauryl sulphonic acid has been used for acids and bases respectively.

Affinity chromatography is similar to ion exchange chromatography. In this technique, a ligand, which binds specifically to one particular compound or a small group of compound, is bonded to an insoluble support such as polystyrene beads or silica. When a complex biological mixture is percolated down a column of such a material, only those compounds, which have affinity for the bonded group, are retained. After elution of the other components of the mixture, changing the mobile phase elutes the retained compounds under analysis. The technique has been used successfully in the separation of enzymes, viruses, and nucleic acids.

The popularity of reverse phase mode is the result of several advantages over normal phase chromatography as the stationary phase is non-polar while the mobile phase is polar, the weakest mobile phase is water, and the strength can be adjusted by adding polar organic solvents. The interactions of the solute with the stationary phase are weak, which provide rapid mass transfer with little chance of irreversible adsorption, and provides rapid equilibration with solvent changes, making the technique ideal for gradient elution methods.

Size exclusion chromatography is also known as gel permeation, gel filtration and steric exclusion chromatography. In size exclusion chromatography the separation occurs on the basis of the difference in the molecular weights and shapes of the solutes. It is unique among all the LC techniques in that a separation is from purely entropic forces. There should be no enthalpic contribution to retention. In other words, interactions such as adsorption, ion exchange and partitioning should be absent in the ideal size-exclusion chromatography. One of the most serious disadvantages of SEC is limited peak capacity, that is only a few separated bands can be accommodated with in the total chromatogram, as all peaks must elute between the total exclusion volume and the total permeation volume.

Ion-exchange chromatography involves the reversible exchange of ions between mobile and stationary phase. The stationary phase has charge bearing functional groups, with the most common mechanism being simple ion exchange. The sample ion is in competition with the mobile phase ion for the ionic sites on the ion exchanger. In HPLC, ion exchangers are generally bonded quaternary ammonium groups for the separation of anions, and a bonded sulphonic acid group for the separation of cations. It is widely used for the separation of nucleic acids, amino acids and sugars.

A typical HPLC instrument consists of -

a) Solvent delivery system including pump
b) Sample injection system
c) Chromatographic column
d) Detector and recording system

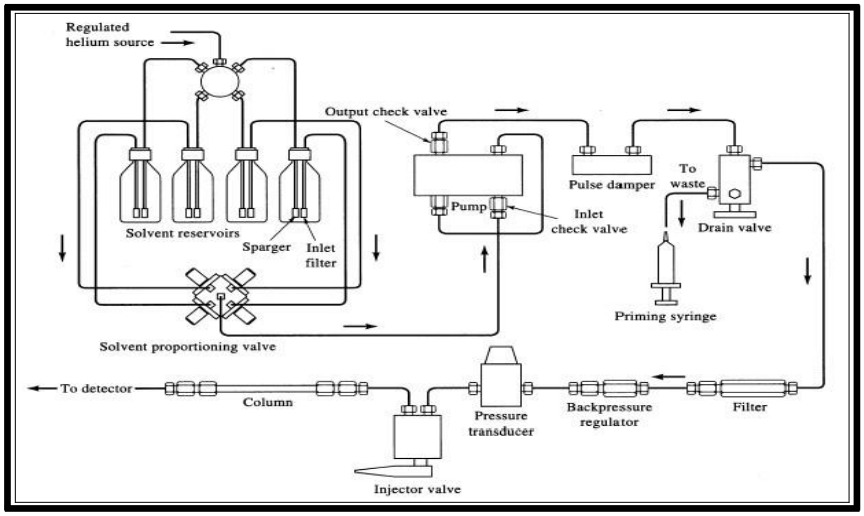


Figure 1: A Schematic diagram of HPLC Equipment

3.1 Pump:

The HPLC pump is considered to be one of the most important components in a liquid chromatography system, which has to provide a continuous constant flow of the eluent through the HPLC injector, column, and detector. The two basic classifications are the constant-pressure and the constant-flow pump. The first is used only for column packing. The second type is the most widely used in all common HPLC applications.

Standard HPLC pump requirement:

Flow rate range: From 0.1 ml to 10 ml per minute.

Presure Range : 1 to 5000 PSI

Presure Pulsation : Less than 1 % for normal and reverse phase mode less than 0.2% for size exclusion mode.

3.1.1 Constant Flow Pumps

Constant-flow systems are generally of two basic types: reciprocating piston and positive displacement (syringe) pumps. The basic advantage of both systems are their ability to repeat elution volume and peak area, regardless of viscosity changes or column blockage, up to the pressure limit of the pump.

Reciprocating piston pump (Fig. 2, Fig. 3) can maintain a liquid flow for indefinitely long time, while a syringe pump has to be refilled after it displaces the whole syringe volume. On the other hand, a syringe pump does not have any flow and pressure pulsations compared to the reciprocating pump. For the micro-HPLC applications a syringe pump allows for the maintaining of a constant flow at the µL/min flow rate range.

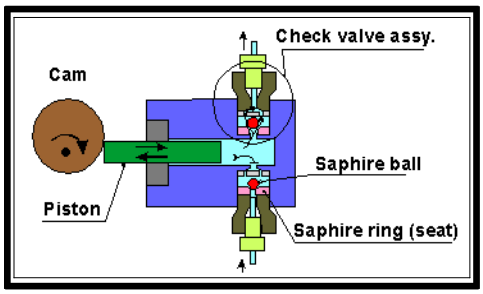


Figure 2: Reciprocating piston pump.

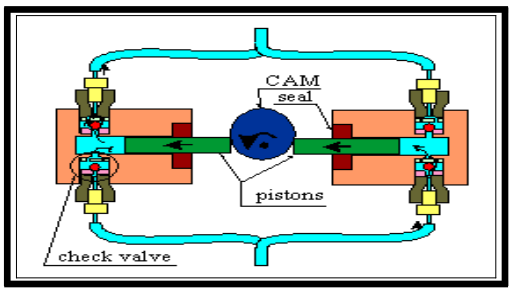


Figure 3: Schematic diagram of Dual head reciprocating pump

3.2 Sample injection system:

Injectors for liquid chromatographic systems should provide the possibility of injecting the liquid sample within the range of 0.1 to 100 mL of volume with high reproducibility and under high pressure (up to the 4000 psi). They should also produce minimum band broadening and minimize possible flow disturbances.

Generally, the most useful and widely used sampling device for modern LC is the microsampling injector valve (Fig. 4).

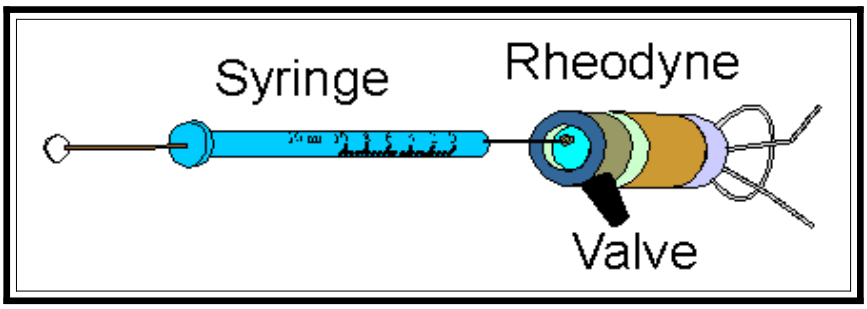


Figure 4: Rheodyne injector

Because of their superior characteristics, valves are now used almost to the exclusion of syringe injection. With these sampling valves, samples can be introduced reproducibly into pressurized columns without significant interruption of flow, even at elevated temperature.

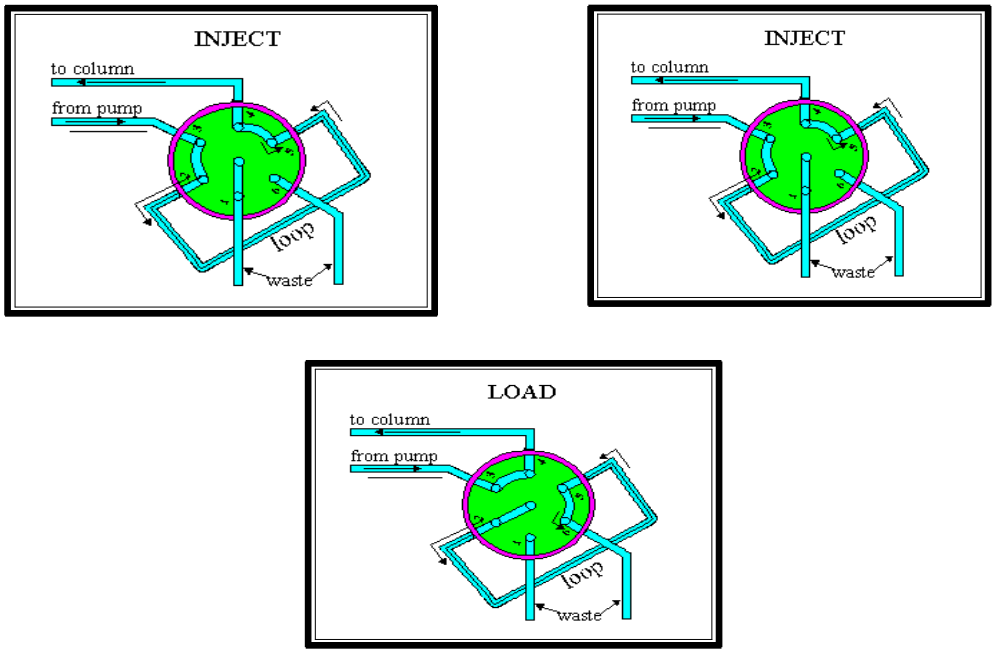


Figure 5: Mechanism of Rheodyne injector

Fig. 5 shows schematic drawings of a six-port Rheodyne valve in which the sample fills an external loop. Compared to shorter, wider i.d. sample loops, long, narrow loops are preferred when large sample volumes are required, because of lesser band-broadening effects. Alternatively, a specially designed syringe may

be used to inject a small volume (e.g., <10 µL) into the loop when required, although in this case the precision in the sample introduction is dependent on the precision of syringe delivery

3.3 Chromatographic column:

Column material is normally Type 316 stainless steel, because it offers the best compromise of cost, workability, and corrosion resistance. Most commercial columns (Fig. 11) available are 10-30 cm long and have internal diameters of either 2.6-3 mm or 4.6-5 mm. The column packing material usually has particle size of 1-10µ. Preparative columns have larger diameters, usually O.D. values are 3/8 - 5/8", corresponding to internal diameters of 6.4-12.7 mm, respectively.

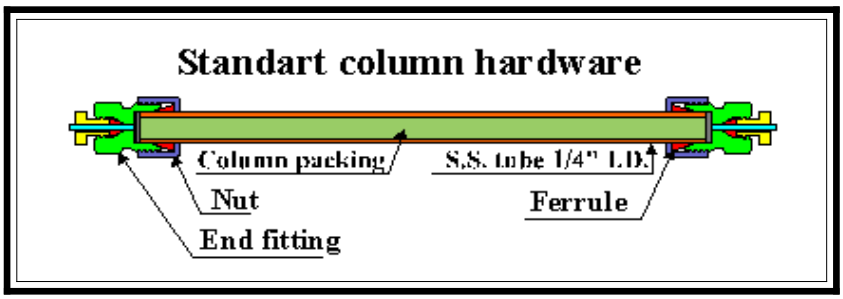


Figure 6: Standard HPLC column

3.4 Detector and recording system:

There are two types of detectors used in HPLC.

a) Bulk property detectors - measure property of mobile phase (refractive index, dielectric constant, density).
b) Solute Property detectors – measures property of solute not present in mobile phase.

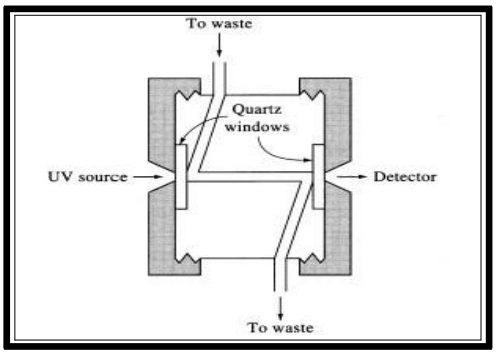


Figure 7: UV-VISIBLE detector for HPLC

3.5 Light sources:

- Single line (arc or hollow cathode lamp, laser)
- Continuum (Xe, D2 lamp)[5].

3.6 Quantitative analysis in HPLC:

Three methods are generally used for quantitative analysis. They are the external standard method, the internal standard method and the standard addition method.

External standard method:

It is the most simple calibration method. It can be used if the recovery of the analyte is 100%and can be expected to be similar for all expected samples. The external standard method involves alternate analysis of standard solutions and of the test solution. By making the use of injections of standard solutions a plot of peak area vs. amount injected be constructed and can be used to quantitate the peak areas from the sample solutions. One can also use the slope of calibration curve based on standard that contain known concentrations of the compound of interest.

Standard addition method:

Standard addition is a useful calibration method when the sample matrix cannot be reproduced well enough to prepare standard solutions for calibration. Here a known amount of the standard compound is added to the sample solution to be estimated. This method is suitable if sufficient amount of the sample is available and is more realistic in the sense that it allows calibration in the presence of excipients or other components.

Internal standard method

A widely used technique of quantification involves the addition of an internal standard to compensate for various analytical errors. In this approach, a known compound of a fixed concentration is added to the known amount of samples to give separate peaks in the chromatograms to compensate for the losses of the compounds of interest during sample pretreatment steps. Any loss of the component of interest will be accomplied by the loss of an equivalent fraction of the internal standard. The accuracy of this approach obviously depend on the fact that internal standard peak be well separated from the peaks of all other components of the sample. This method is used for the analysis of biological samples and other more difficulty.

3.7 Design and development of HPLC method:

The most important factor to be considered in developing a HPLC method for analysis of drugs or analytes in multicomponent system is the solubility of the analytes in the mobile phase. The prediction of solubility can be made by careful

study of molecular properties like, molecular weight, ionic characters, and polarity of the molecules. The stationary phase selection is also equally important as mobile phase selection.

The following figure gives an overview about the best possible selection of stationary andmobile phase for separation of analytes depending on the molecular properties.

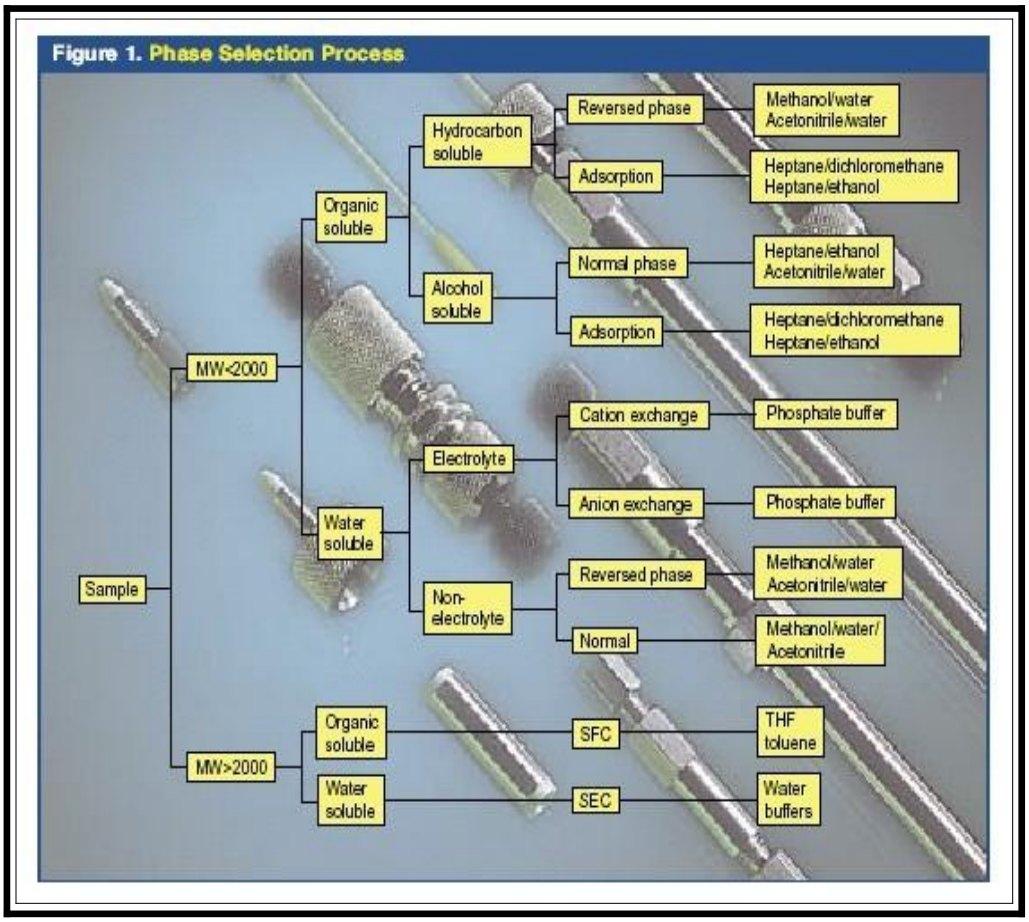


Figure 8: The most appropriate stationary and mobile phase for analyte.

- By getting the primary information about the molecular properties of the analytes we can get some idea about the design of the proposed method, but usually the method development depends on trial and error bases. The basic problem in HPLC method development is selection of stationary and mobile phase. People usually preferred reverse phase HPLC method for separation of analytes if they are ionisable and water-soluble with many polar groups.
- The next thing considered for developing a method of analysis by RP-HPLC is the selection of detector. The detector selected should be such that it gives very good response with in the concentration range of analytes. Table 1 gives a brief

idea about the type of detector used for method development depending on the property of the analyte and mobile phase. When the analytes having ionisable groups are ought to be analyzed by RP-HPLC then one should consider the appropriate selection of buffer in mobile phase as pH of the mobile phase is highly affecting the separation. In reversed phase HPLC, the retention of analytes is related to their hydrophobicity. The more hydrophobic the analyte, the longer it is retained. When an analyte is ionized, it becomes less hydrophobic and, therefore, its retention decreases. Acids lose a proton and become ionized when pH increases and bases gain a proton and become ionized when pH decreases. Therefore, when separating mixtures containing acids and/or bases by reversed phase HPLC, it is necessary to control the pH of the mobile phase using an appropriate buffer in order to achieve reproducible results. . In general, most buffers should provide adequate buffering capacity for controlling mobile phase pH only within ± 1 unit of their pKa. The pH of the mobile phase has to be selected in such a way that the compounds are not ionized. If the retention times are too short, the decrease of the organic phase concentration in the mobile phase can be in steps of 5%. If the retention times are too long, an increase of the organic phase concentration is needed.

- The next important consideration in RP-HPLC method development is the selection of analytical wavelength, in case of analytes containing chromophoric groups, detected by UV-VISIBLE detector, for this several solutions of individual analyte has to be made and scanned in UV or VISIBLE region for the λ_{max} selection. The analytical wavelength selected should be such that all the analytes of our interest shows good absorption at that particular wavelength.

Table 1: Selection of detector based on molecular properties:

Detector	*Analytes*	*Solvent Requirements*	*Comments*
UV-Visible	Any with Chromophores	UV-grade non-UV absorbing solvents	Has a degree of selectivity and is use- ful for many HPLC applications
Fluorescence	Fluorescent compounds	UV-grade non-UV absorbing solvents.	Highly selective and sensitive. Often used to analyze derivatized compounds

Refractive Index (RI)	Compounds with a different RI to the mobile phase	Cannot run mobile phase gradients	Virtually a universal detector but has limited sensitivity
Conductivity	Charged or polar compounds	Mobile phase must be conducting	Excellent for Ion Exchange methods
Electrochemical	Readily oxidized or reduced compounds, especially biological Samples	Mobile phase must be conducting	Very selective and sensitive
Evaporative Light Scattering (ELSD)	Virtuall all compounds	Must use volatile solvents and volatile buffers	A universal detector, which is highly selective but not sensitive.
Mass Spectrometer (MS)	Broad range of compounds	Must use volatile solvents and volatile buffers	Highly sensitive and is a powerful 2^{nd} dimensional analytical tool.Many modes available.Needs trained operator.

When all the primary parameters like stationary phase, mobile phase and detector has been decided, one should go for actual recording of the chromatograms. While recording the chromatograms one should get the different peak for different analytes. If one is not getting that, then the parameters of analysis like mobile phase flow rate, composition of mobile phase etc should be changed. When tailing or fronting is observed, it means that the mobile phase is not totally compatible with the solutes. In most case the pH is not properly selected and hence partial dissociation or protonation takes place. When the peak shape does not improve by lower (1-2) or higher (8-9) pH, then ion-pair chromatography can be used. For acidic compounds, cationic ion pair molecules at higher pH and for basic compounds, anionic ion-pair molecules at lower pH can be used. For amphoteric solutes or a mixture of acidic and basic compounds, ion-pair chromatography is the method of choice.

The low solubility of the sample in the mobile phase can also cause bad peak shapes. It is always advisable to use the same solvents for the preparation of sample solution as the mobile phase to avoid precipitation of the compounds in the column or injector.

Optimization can be started only after a reasonable chromatogram has been obtained. A reasonable chromatogram means that more or less symmetrical peaks of all the compounds are detected on the chromatogram. By making the slight change of the mobile phase composition, the position of the peaks can be predicted within the range of investigated changes. An optimized chromatogram is the one in which all the peaks are symmetrical and are well separated in less run time.

The peak resolution can be increased by using a more efficient column (column with higher theoretical plate number, N) which can be achieved using a column of smaller particle size, or a longer column. These factors, however, will increase the analysis time. Flow rate does not influence resolution, but it has a strong effect on the analysis time.

The parameters that are affected by the changes in chromatographic conditions are

a. Capacity factor (k)
b. Selectivity or Relative Retention (RRT)
c. Column efficiency (N)
d. Resolution (R_S)
e. Peak asymmetry factor (A_S)

Capacity Factor (k'):

The Capacity Factor, k, of a sample component is a measure of the degree to which that component is retained by the column relative to an unretained component. The capacity factor is also known as Retention Factor or Relative Retention.

$$K = (T_R - T_0)/T_0$$

Where T_R = the elution time of retained compound.

T_0 = the elution time of unretained compound.

Selectivity (α):

$$\alpha = K_B/K_A$$

Separation factor gives the degree of separation of two components under a given set of conditions. The separation factor is defined as the ratio of the capacity factors. The higher the value of α better is the separation.

Column efficiency:

Efficiency (N) of a column is the measure of the number of theoretical plates per meter. It is a measure of band spreading of a peak. Smaller the band spread, higher is the number of theoretical plates, indicating good column and system performance. Columns with N ranging from 5,000 to 100,000 plates/meter are ideal for a good system. Efficiency is calculated by the following equation.

$$N=16\ T_R^2 / W^2$$

Where T_R = retention time and
W = peak width.

Resolution (R_S):

Resolution (Rs) characterizes the ability of a chromatographic column to separate two analytes. It is the difference between the retention times of two solutes divided by their average peak width.

$$Rs = (1/4)(\alpha - 1)(N)^{1/2}[k/(1+k)]$$

Where, k = average value for the two peaks

N = number of theoretical plates
α = selectivity of the system
The term can be modified by changes in the mobile phase. The number of theoretical plates can be affected by changing the length of the column, the flow rate or the column packing material k can be changed by changing the solvent strength of the mobile phase. For complete resolution the value of RS 1.5 is achievable.

Peak symmetry factor:

Peak asymmetry factor, also known as tailing factor (T) can be used as a criterion of column performance. It can be calculated as

$$T=W_{0.05}/2f$$

Where,
$W_{0.05}$ = Width of peak at 5% height.
f = Distance from the peak maximum to the leading edge of the peak, the distance being measured at a point 5% of the peak height from the base line[6].

Chapter 4

LITERATURE REVIEW

4.0 LITERATURE REVIEW:

The literature review reveals that a less amount of work has been done on Naftopidil and its Tablets formulation. There are few stability indicating method reported for the same in its tablets dosage form. The drug is non pharmacopoeial and hence more and more research is welcome on it. There are few methods reported in the literature.

- **F. Behn *et.al..*[7]**. reported an HPLC method for Carvedilol in plasma of small children, where Naftopidil is used as an internal standard. The two drugs were analyzed by solid phase extraction and were eluted using Acetonitrile: Acetate Buffer (0.1M, pH 5.0). Further the extract was analyzed on fluorescence detection.
- **M. Yuan and co worker[8]**. have reported a method for the separation of enantiomers of Naftopidil. They developed a chiral HPLC method for the separation and analysis of naftopidil enantiomers. The two enantiomers of Naftopidil were separated using a Chiralpak AD-H (250 mm×4.6 mm, 5 μm) column and monitored at the wavelength of 283 nm. The isocratic mobile phase consisting of hexane–isopropanol–Diethylamine (85:15:0.1, v/v/v) was pumped at a flow rate of 1.0 mL/min. Under these chromatographic conditions, *R*-Naftopidil and *S*-Naftopidil were well separated and had good linearity in the ranges of 0.78–50 μg/mL (r = 0.9999) and 0.84–54 μg/mL (r = 0.9998), respectively. The relative standard deviations (RSD) of intra- and inter-day assays were no more than 0.5% and 0.7%, respectively. This improved method for the separation and quantitative determination of Naftopidil enantiomers can be used for the quality control of synthesized Naftopidil product.
- **S. Pritam Jain *et.al.*[9]**. studied a method for Naftopidil in Human Plasma by LC-MS/MS. The chromatographic condition for separating the Naftopidil by using Discovery C18 column (50 mm,4.6 mm, 5 μ) maintained at 40^{O} C . The LC mobile phase consisted of methanol: ammonium formate (90:10, v/v). The flow rate was 0.5 mL/min. The injection volume was 10μL, and the runtime was 3.0 min. They used a Concentration range of 5–150 lg/mL and correlation coefficient was 0.999 and provide a signal-to-noise ratio of 3:1 which was found to be 0.19.

  Propranolol is used as the internal standard. The lower limits of quantification are 0.495 ng/mL. The calibration curves are linear over the concentration range of 0.495–200.577 ng/mL of plasma for each analyte. This novel LC–MS/MS method shows satisfactory accuracy and precision and is sufficiently sensitive for the performance of pharmacokinetic studies in humans
- **G. Niebch *and co author*[10]** reported the metabolism of Naftopidil in animals and humans revealed two major metabolites, 0-desmethyl Naftopidil and (phenyl) hydroxyl Naftopidil (unpublished observatrons), each with an affinity for alfa 1-adrenoceptors similar to that of the parent compound .They describes a rapid high-performance liquid

chromatographic (HPLC) assay with fluorescence detection for the quantitative determination of Naftopidil and its two active metabolites in blood plasma, using an internal standard (Carvedilol).

The Chromatographic condition was

Columns (250 mm x 4.6 mm I.D.) packed with 5-pm RP-LiChrosorb Select B (Hibar) .

The mobile phase components were 0.02 M KH_2PO_4 (adjusted to pH 1.8 with phosphoric acid) (A) and acetonitrile-methanol (1: 1, v/v) (B). They were mixed in a ratio A:B = 55:45.

The flow-rate was 0.8 ml/min.

Fluorescence detector was used with the following settings: excitation wavelength 215 nm; emission wavelength above 320 nm.

The assay results obtained from the above analysis follow the validation protocol with a linearity range in a concentration between 1-150 µg /ml .

- **N. Satheeshkumar *et.al.*[11]** reported a HPLC process to estimate the Naftopidil in its bulk and pharmaceutical dosage form. Separation was achieved using a C18 GRACE column (250 mm × 4.6 mm, 5 µm particle size) and gradient mobile phase system consisting of (A)10 mM of ammonium acetate buffer pH adjusted to 4.0 with glacial acetic acid and (B) acetonitrile. The flow rate was 1.0 mL/min with UV detection at 284 nm. Naftopidil was subjected to stress conditions like hydrolysis (acid, alkali and neutral degradation), oxidation, photolytic and thermal decomposition. The linearity of the proposed method was investigated in the range of 10-150µg/mL. Application of design of experiments for the robustness study method was carried out, where in five factors was selected: pH of mobile phase, flow rate, strength of the buffer and column temperature. The analytical method for NAF was developed and validated at the linearity range of 10-150 µg/mL. The LOD and LOQ were 0.6 and 2.04, respectively and accuracy of analysis was 100.5-101.1%.

- **B. Pavan Adithya *et.al.*[12]** investigated a new simple, precise, sensitive and validated RP-HPLC method for the estimation of Naftopidil in pharmaceutical dosage form. The chromatographic conditions used for the separation was Phenomenex Luna C8 (4.6x150mm, 5µ) and mobile phase comprised of Methanol: Water (90:10 v/v). The flow rate was 0.8 ml/min with detection at 232 nm. The retention time was found to be 2.85 min. The linearity was found to be in the range of 1 - 5µg/ml with correlation coefficient of 0.9998. %RSD of repeatability, intraday and inter day variations were found 0.77, 0.59-1.32, 0.63-1.72). The limit of detection (LOD) and limit of quantitation (LOQ) were found to be 0.07552 and 0.2288 µg/ml respectively.

- **K. Raghu Babu *et.al*.**[13] reported a new simple, accurate, precise, sensitive and validated RP-HPLC method for the estimation of Naftopidil in bulk and pharmaceutical dosage form. The Chromatographic conditions used for the separation was Zorabax SBC18 (150x4.6 MM, 5µ) and the mobile phase comprised of Acetonitrile and Ammonium Acetate (75:25 v/v). The flow rate was 1.2 mL/min. The detection was carried out at 232 nm. The Assay method was validated as per ICH guidelines. The linearity was found to be in the range of 0.1 – 0.6 mg/ml (25% to 150%) with correlation coefficient(r) 0.9946. The proposed method is accurate with 99.77% - 99.98% recovery for Naftopidil and precise. (%RSD of repeatability, intraday and inter day variations were 0.267 - 0.621

Chapter 5

AIM OF THE WORK

5.0 AIM OF THE PRESENT WORK:

- Literature survey does not reveal any method for estimation of Naftopidil in pharmaceutical dosage form, therefore it was thought of interest to develop simple, sensitive, accurate, precise, rapid analytical method for estimation of this drug.

- To develop and validate high performance liquid chromatography (HPLC) method for Naftopidil in bulk and dosage form.

- Both the developed methods to be validated statistically to ensure their accuracy, precision, repeatability, and reproducibility and other analytical method validation parameters as mentioned in the ICH guideline.

- To develop Stability Indicating method by HPLC To prove the method to be stability indicating by carrying out forced degradation studies and validate the proposed method as per ICH guidelines.

Chapter 6

EXPERIMENTAL

6.0 EXPERIMENTAL:

6.1 Materials and reagents:

Naftopidil API (B.No.-131201) as gift sample was obtained from SUN Pharma Sikkim, India and NAFODIL 50® containing 50 mg (B.No.-KR1983) Naftopidil per tablet, was procured from market manufactured by Intas Pharmaceuticals (Sikkim, India). LC grade methanol and acetonitrile were purchased from Merck. Analytical Reagent grade Potasium dihydrogen orthophosphate and Othophosphoric acid were procured from Merck (Mumbai, India). The Ultra pure water from Rankem was used throughout the study. All other chemicals in this study were of analytical grade.

6.2 Instrumentation:

For the development of HPLC method High performance liquid chromatography equipped with Waters 515 pump, manual injector and 2489 UV detector with pump control module (PCM) and Empower 2 software was utilized. Other instrument like pH meter from E.M.Instrument (EM - 8), Balance Metlar Toledo and ultra sonicator were used. All instrument and glass ware is calibrated.

6.3 Preparation of Buffer:

Weighed 6.8 grams of Potassium di hydrogen ortho phosphate into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC grade water and pH adjusted to 3.5 with Orthophosphoric acid.

6.4 Preparation of Mobile Phase:

Mobile was prepared by mixing 50 volume of buffer and 50 volumes of acetonitrile and filter through 0.2 micron filter paper.

6.5 Diluent:

Methanol

6.6 Preparation of Standard Solution:

Accurately weighed and transferred 100 mg of Naftopidil working standard into a 100 ml volumetric flask, about 70 ml of methanol was added and sonicated to dissolve it completely and volume was made up to the mark with the methanol 100 mcg/ml (**Stock solution**). 0.3 ml of the above stock solution was pipette into a 10 ml volumetric flask and diluted up to the mark with methanol. Mixed well and filtered through 0.45µm filter.

6.7 Preparation of Sample Solution:

About 10 Naftopidil tablets were weighed and average weight was calculated (Weight of 10 Tablet = 1.5033 gm, Avg. Wt = 150.33 mg). Accurately weighed and transferred the sample equivalent to 100 mg of Naftopidil into a 100 ml volumetric flask. About 70 ml of diluents was added and sonicate to dissolve it completely and volume was made up to the mark with diluents. Mixed well and filtered through 0.45µm filter. Further pipette 0.3 ml of the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluents. Mixed well.

6.8 Method development & Optimization by HPLC:

- Equipment : HPLC Waters
- Column : Stainless Steel column packed with Octadecylsilane porous silica such as BDS Hypersil (C_{18}) 250 mm X 4.6 mm, 5µ.Make by Thermoscintific.
- Flow rate : 1.0 ml/min
- Wavelength : 282 nm
- Injection Volume : 20 µl
- Temparature : Ambient
- Run Time : 10.0 min.

6.9 Estimation of Naftopidil by HPLC:

About 20 µl of the standard and sample were injected into the chromatographic system and measure the area of the Naftopidil peak and % assay was calculated by using the formulae (See Appendix chapter; page no.-32 to 36).

6.10 Validation of HPLC method:

6.10.1 Linearity:

Aliquots of 0.1,0.2,0.3,0.4,0.5 ml standard **stock solution** (100 µg/ml) was transferred to the 10 ml of volumetric flasks and made up to the mark with diluents to get a concentration of 10,20,30,40,50 µg/ml . An aliquot (20 µl) of each solution was injected under the operating chromatographic condition as described above and responses were recorded. Calibration curve was constructed by plotting the peak areas versus the concentration and the regression equation was calculated by software (See Appendix chapter; page no.-39 to 44). The fixed standard solution was prepared by transferring 0.3 ml of Naftopidil (100 µg/ml) to 10 ml of volumetric flask and made up to the mark with diluents to get 30 µg/ml of Naftopidil.

6.10.2 System Suitability:

20 µL of the standard solution (30 µg/ml) was injected under optimized chromatographic conditions to evaluate the suitability of system. (Appendix chapter; page no.-37 to 44)

6.10.3 Precision:

The precision of the method was determined by repeatability and intermediate precision (intra-day and inter-day).

Repeatability

The Repeatability of the proposed method was ascertained by injecting six replicates of fixed concentration within the Beer's range and finding out the peak area by the proposed method. From this peak area %RSD was calculated. (Table: 2) (Appendix, page no 44)

Intra-day precision

Intra–day precision was determined by injecting three different concentrations (90 %, 100% and 110%) for three times in the same day. Peak area was measured and %RSD was calculated. (Table: 2) (See Appendix chapter; page no.-51 -53)

Inter-day precision

Inter-day precision was determined by injecting same concentrations (30mcg/ml) for three days in a week. Peak area was measured and %RSD was calculated. (Table: 2) (See Appendix chapter; page no.-54 to 55)

Table 1: Validation Parameter Data

Linearity (µg/ml)	10-50
Normalized Intercept(a)/ Slope(b)	0.025360
Correlation co efficient (r)	0.997015
Correlation co efficient (r^2)	0.994038
Repeatability(%RSD, n=6)	1.4
Interday precision(%RSD ,n=3)	0.4 – 1.1
Regression equation(y=mx+c)	Y=2.81e+004X + 2.14e+004
Intraday precision(%RSD n=3)	0.9 - 1.4
% Recovery	99.73%
Robustness	Robusted
LOQ(µg/ml)	3.8405
LOD(µg/ml)	1.2673

6.10.5 Accuracy:

For the accuracy of proposed method, recovery studies were performed by standard addition method at three different levels (50%, 100% and 150% of final concentration).A known amount of standard pure drug was added to pre-analyzed tablet powder and the sample was then analyzed by proposed method. Results of recovery studies were found to be satisfactory and reported in Table 2 (See Appendix chapter; page no.-59 to 65)

Table 2: Accuracy Data

Drug Name	Level	Amaount Added (mg/ml)	Recovery %	Mean Recovery
Naftopidil	50%	0.150	99.26%	99.73 %
Naftopidil	100%	0.312	99.65%	
Naftopidil	150%	0.462	100.3%	

6.10.6 Limit of Detection and Limit of quantitation:

Limit of detection (LOD) and Limit of Quantification (LOQ) were determined by using the formula based on the standard deviation of the response and the slope.LOD and LOQ were calculated by using equations, **LOD=3.3 X δ/s and LOQ=10 X δ/s**, where δ = standard deviation, s = slope of the calibration curve.(Table 2). The middle concentration (30mcg/ml) is selected for LOD and LOQ calculation.

6.10.7 Robustness:

The robustness of the HPLC method was evaluated by analyzing the system suitability parameters after varying the Flow rate (±0.2), organic solvent content (± 5%),. None of these alterations caused a significant change in peak area RSD, tailing factor and theoretical plates. Although the changes in the retention time were significant, yet quantitation was possible. The results were tabulated in Table 2. (See Appendix chapter; page no.-67 to 68).

6.11 Stability indicating method:

Stress conditions were decided on the basis of tolerable pH range of the column, and some other stability indicating assays published in the literature[15]. Resolution of between drug and its degradants peak should be more than 1.5 reported in the literature (Analytical Method Validation, by G.M. Green *at* al'). Attempt was made to decompose 10-30% of the drug by exposing drug to stress conditions and then milder conditions were used. This was done to reduce the time of degradation. The tolerable pH range of column is 2.5-8.5 therefore higher alkaline stress conditions cannot be used. Here higher concentration of sample and standard were used to find out either the sample is decomposed or not. Hence only single injection was madealong with a blank injection.No quantitation done.

6.11.1 As such condition:

In this the solutions are prepared without any treatment on blank, standard and sample. In this 100 mg API and 300 mg crushed tablets of samples are taken in two 100 ml volumetric flask, add 60 ml of diluents, sonicate for 10 min, and then make up volume with diluents. Then filter the solutions through 0.45 µm filter and use this solution for experiment.

6.11.2 Acidic condition:

Drug was subjected to acidic condition to achieve degradation from 10 – 30%. 300mg API, and 600 mg crushed tablets were suspended with 10 ml of 1N hydrochloric acid separately, then these solutions were kept for 24 hrs. After that suspension was

filtered and washes with water and dries at a temperature 105°C, from that sample proceeds as directed in the 6.11.1 and use the supernatant liquid for experiment.

6.11.3 Alkaline condition:

Drug was subjected to alkaline condition to achieve degradation from 10 – 30%. 300 mg API, and 600 mg crushed tablets were suspended with 10 ml of 1N sodium hydroxide solution separately, then these solutions were kept for 24 hrs. After that suspension was filtered and washes with water and dries at a temperature 105°C. That sample proceeds as directed in the 6.11.1 and use the supernatant liquid for experiment.

6.11.4 Oxidation condition:

Drug was exposed to oxidizing medium through hydrogen peroxide solution. Attempt was taken to achieve degradation between 10.0 – 30.0%. 300 mg API, and 600 mg crushed tablets were suspended with 5 ml of 5% hydrogen peroxide solution, then these solutions were kept for 24 hrs. After that suspension was filtered and washes with water and dries at a temperature 105°C. That sample proceeds as directed in the 6.11.1 and use the supernatant liquid for experiment..

6.11.5 Exposed to UV radiation:

API and tablets were exposed to UV radiation for 24 hrs. Then 100mg API, and 300 mg crushed Tablets were dissolved with 60 ml of Diluents, sonicate for 15 min. The volume was made up TO 100 ml with diluents and proceeds as directed 6.11.1.

6.11.6 Thermal Degradation:

API, Blank, and tablets were exposed to dry heat for 1 days. Then 100mg API, and 300 mg crushed tablets were dissolved with 60 ml of diluents, sonicate for 15 min. The volume was made up to 100 ml with mark with diluents and proceeds as directed 6.11.1.

6.11.7 Humidity Degradation:

API, and tablets were kept in stability chamber (Newtronic) a condition 30°C temperature and 60% RH for 1 month. Then 100 mg API, and 300 mg crushed Tablets were dissolved with 60 ml of Diluents, sonicate for 15 min. The volume was made up to 100 ml with diluents, then proceeds as directed in 6.11.1.

Table 3: Summary of data of Stability Indicating Method by HPLC

Serial No.	Sample	Condition	% of secondary peak area	% Naftopidil peak
1	Blank	As Such	-	-
2		Acidic	-	-
3		Alkaline	-	-
4		Peroxide	-	-
5		UV light	-	-
6		Thermal	-	-
7		Humidity	-	-
8	API	As Such		100
9		Acidic		100
10		Alkaline		100
11		Peroxide		100
12		UV light		100
13		Thermal		100
14		Humidity		100
15	Tablet	As Such		100
16		Acidic		100
17		Alkaline		100
18		Peroxide		100
19		UV light		100
20		Thermal		100
21		Humidity		100

6.12 Result & Discussion:

To develop simple and economical RP-HPLC method, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was obtained with BDS Hypersil C18 (250 x 4.6 mm, 5µ) column and mobile phase comprising of Acetonitrile : Buffer (50:50 v/v) at a flow rate of 1.0 ml/min to get better reproducibility and repeatability. Quantification was achieved with UV detection at 282 nm based on peak area. The retention time was found to be 5.871 min. The optimized method was

validated as per ICH guidelines. The system suitability parameters observed by using this optimized conditions were reported in Appendix chapter; page no 45. A linearity range of 10-50 µg/ml with correlation coefficient 0.997015 was established. The result of recovery study by standard addition method ranging from 99.26 % to 100.3 % suggested good accuracy. The precision of the proposed method was checked in terms of the repeatability, inter-day and intra-day time periods. The low % RSD values of repeatability (1.4%), inter-day (0.9% - 1.4%) and intra-day (0.4 % – 1.1 %) variations reveal that the proposed method is precise. The LOD, LOQ values were found to be 1.2673 µg/ml and 3.8405 µg/ml respectively. The absence of interference peak indicates that method can be used for routine analysis of Naftopidil in pharmaceutical dosage form.

6.13 Conclusion:

From the above discussion it can be concluded that as the results of the analysis were validated statistically and all the validation parameters were found to be within the acceptable range, it proves the precision, sensitivity, accuracy, ruggedness, robustness and applicability of the proposed method for the routine quantitative determination of Naftopidil in bulk drug and its dosage form

Chapter 7

SUMMARY

7.0 *SUMMARY :*

- HPLC method was developed for estimation of Naftopidil in Pharmaceutical Dosage form.

- The developed HPLC method was validated for Linearity, Accuracy, Interday Precision, Specificity & Selectivity, Robustness, Solution stability, limit of detection & limit of quantification.

- Stability Indicating Methods were developed for Naftopidil in Pharmaceutical Dosage form.

- Stability Indicating Methods were developed for Naftopidil in Pharmaceutical Dosage form under hydrolytic stress condition (5N HCL, 5N NaOH); Oxidation condition (5% H_2O_2) and dry heat condition, Thermal condition, UV light. From study, it was found that drug is not susceptible for degradation to hydrolytic condition, oxidation condition, dry heat, UV light, and thermal condition.

- All the developed methods were successfully applied to determine the drugs in Pharmaceutical preparation.

Chapter 8

APPENDIX

Component Summary For Retention Time
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil Assay Std 01	1	W2489 ChA	8	5.864
2	Naftopidil Assay Std 02	2	W2489 ChA	8	5.919
3	Naftopidil Assay Std 03	3	W2489 ChA	8	5.878
4	Naftopidil Assay Std 04	4	W2489 ChA	8	5.862
5	Naftopidil Assay Std 05	5	W2489 ChA	8	5.870
Mean					5.879
Std. Dev.					0.024
% RSD					0.4

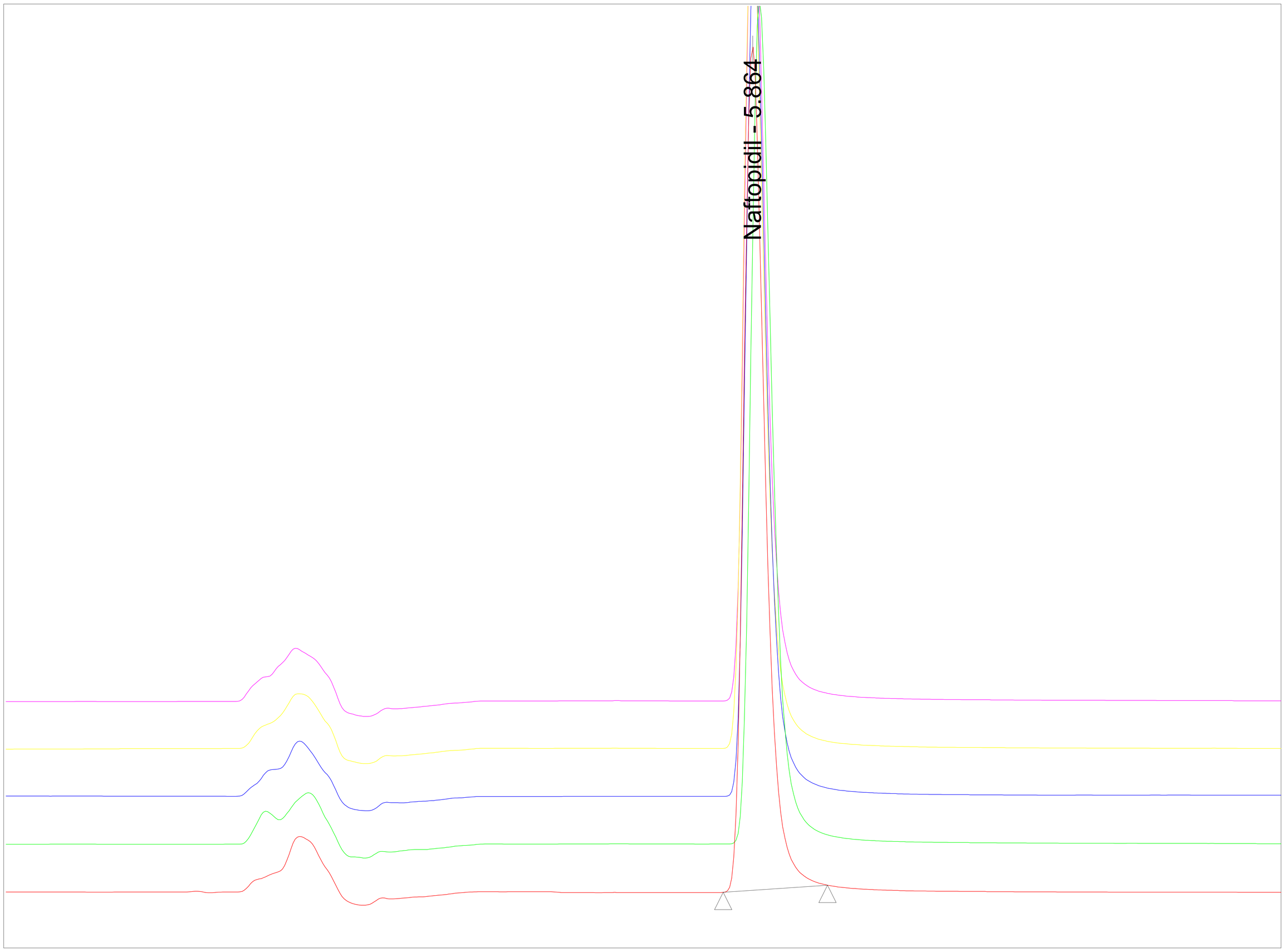
Component Summary For Area
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil Assay Std 01	1	W2489 ChA	8	875121
2	Naftopidil Assay Std 02	2	W2489 ChA	8	883601
3	Naftopidil Assay Std 03	3	W2489 ChA	8	878671
4	Naftopidil Assay Std 04	4	W2489 ChA	8	902416
5	Naftopidil Assay Std 05	5	W2489 ChA	8	879602
Mean					883882
Std. Dev.					10792
% RSD					1.2

Component Summary For Amount
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil Assay Std 01	1	W2489 ChA	8	50.000
2	Naftopidil Assay Std 02	2	W2489 ChA	8	50.000
3	Naftopidil Assay Std 03	3	W2489 ChA	8	50.000
4	Naftopidil Assay Std 04	4	W2489 ChA	8	50.000
5	Naftopidil Assay Std 05	5	W2489 ChA	8	50.000
Mean					50.000
Std. Dev.					0.000
% RSD					0.0

Peak Summary Report



Sample Name: Naftopidil Assay Std 01; Date Acquired: 4/3/2016 4:33:08 PM IST; Vial: 8; Injection: 1
Sample Name: Naftopidil Assay Std 02; Date Acquired: 4/3/2016 4:47:34 PM IST; Vial: 8; Injection: 2
Sample Name: Naftopidil Assay Std 03; Date Acquired: 4/3/2016 4:58:53 PM IST; Vial: 8; Injection: 3
Sample Name: Naftopidil Assay Std 04; Date Acquired: 4/3/2016 5:10:29 PM IST; Vial: 8; Injection: 4
Sample Name: Naftopidil Assay Std 05; Date Acquired: 4/3/2016 5:21:34 PM IST; Vial: 8; Injection: 5

Peak Summary with Statistics
Name: Naftopidil

	Sample Name	Vial	Inj	Name	Retention Time (min)	Area	% Area	Height	Amount	Units
1	Naftopidil Assay Std 01	8	1	Naftopidil	5.864	875121	100.00	74897	50.0	mcg
2	Naftopidil Assay Std 02	8	2	Naftopidil	5.919	883601	100.00	74731	50.0	mcg
3	Naftopidil Assay Std 05	8	5	Naftopidil	5.870	879602	100.00	75576	50.0	mcg
4	Naftopidil Assay Std 04	8	4	Naftopidil	5.862	902416	100.00	77451	50.0	mcg
5	Naftopidil Assay Std 03	8	3	Naftopidil	5.878	878671	100.00	75342	50.0	mcg

Peak Summary with Statistics
Name: Naftopidil

	Sample Name	Vial	Inj	Name	Retention Time (min)	Area	% Area	Height	Amount	Units
Mean					5.879					
Std. Dev.					0.024					
% RSD					0.40					

Component Summary For Retention Time
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil tablet Assay Spl 01	1	W2489 ChA	9	5.873
2	Naftopidil tablet Assay Spl 02	2	W2489 ChA	9	5.871
Mean					5.872
Std. Dev.					0.001
% RSD					0.0

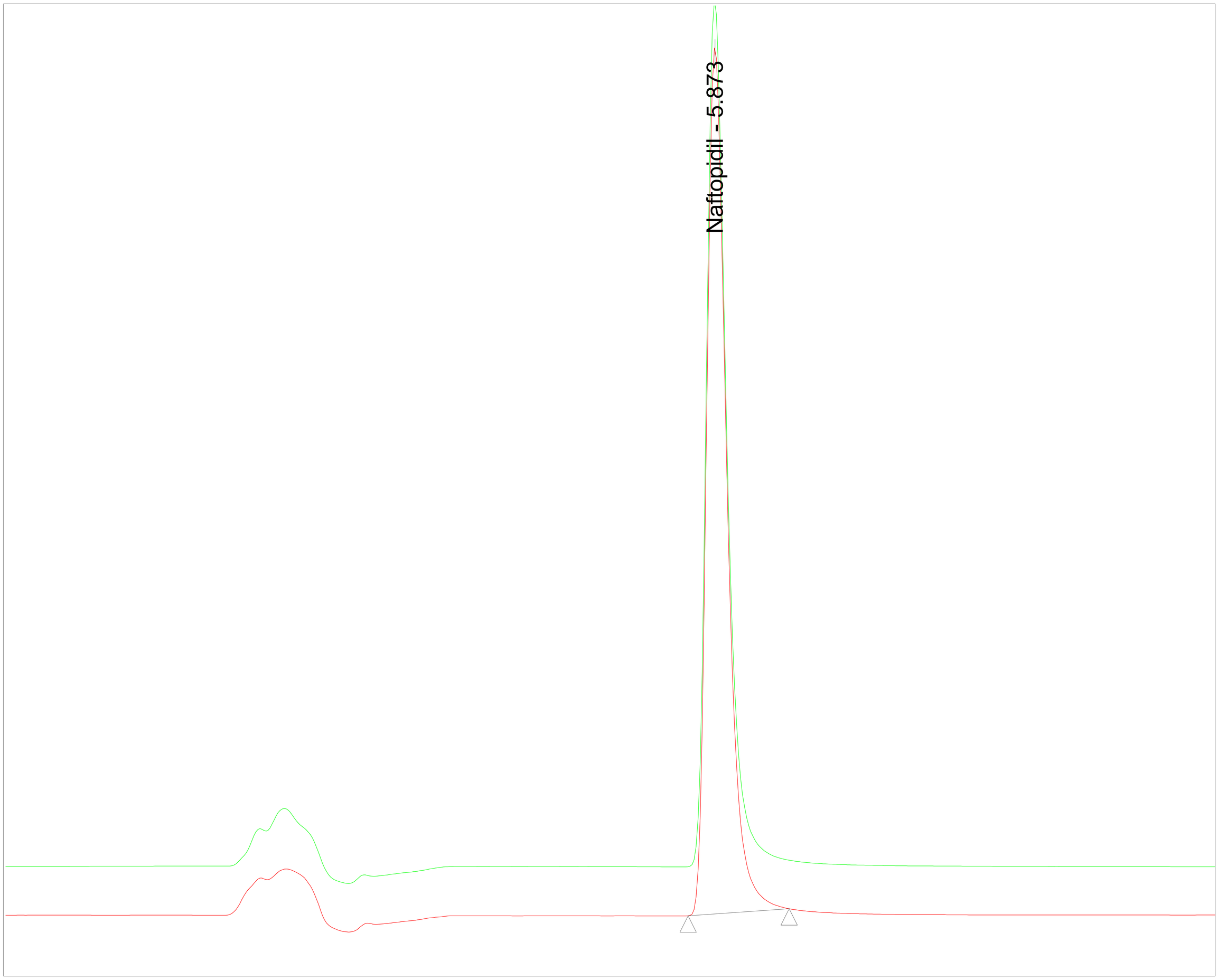
Component Summary For Area
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil tablet Assay Spl 01	1	W2489 ChA	9	922709
2	Naftopidil tablet Assay Spl 02	2	W2489 ChA	9	900842
Mean					911776
Std. Dev.					15462
% RSD					1.7

Component Summary For Amount
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil tablet Assay Spl 01	1	W2489 ChA	9	48.372
2	Naftopidil tablet Assay Spl 02	2	W2489 ChA	9	46.540
Mean					47.456
Std. Dev.					1.296
% RSD					2.7

Peak Summary Report



Sample Name: Naftopidil tablet Assay Spl 01; Date Acquired: 4/3/2016 5:36:18 PM IST; Vial: 9; Injection: 1
Sample Name: Naftopidil tablet Assay Spl 02; Date Acquired: 4/3/2016 5:47:19 PM IST; Vial: 9; Injection: 2

Peak Summary with Statistics
Name: Naftopidil

	Sample Name	Vial	Inj	Name	Retention Time (min)	Area	% Area	Height	Amount
1	Naftopidil tablet Assay Spl 02	9	2	Naftopidil	5.871	900842	100.00	78241	46.5
2	Naftopidil tablet Assay Spl 01	9	1	Naftopidil	5.873	922709	100.00	79401	48.4
Mean					5.872				
Std. Dev.					0.001				
% RSD					0.02				

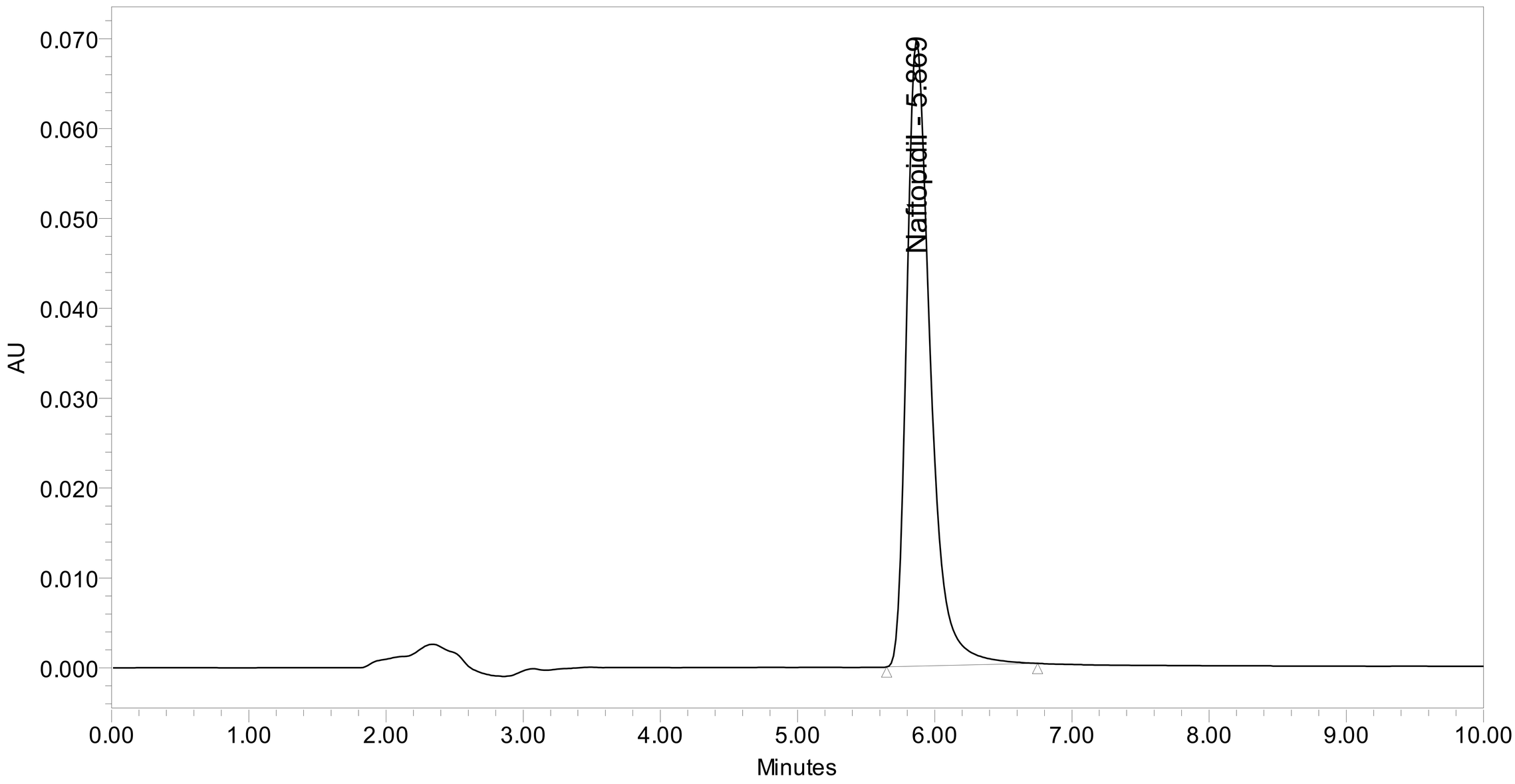
Peak Summary with Statistics
Name: Naftopidil

	Units
1	mcg
2	mcg
Mean	
Std. Dev.	
% RSD	

System Suitability Report

SAMPLE INFORMATION

Sample Name:	Naftopidil SST_30mcg_Inj. 1	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	
Vial:	7	Acq. Method Set:	Naftopidil
Injection #:	1	Processing Method:	Naftopidil
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 282nm
Date Acquired:	4/3/2016 2:43:51 PM IST		
Date Processed:	4/3/2016 4:43:34 PM IST		



	Peak Name	RT	Area	% Area	Height	Amount	Units
1	Naftopidil	5.869	840804	100.00	69794	50.000	mcg

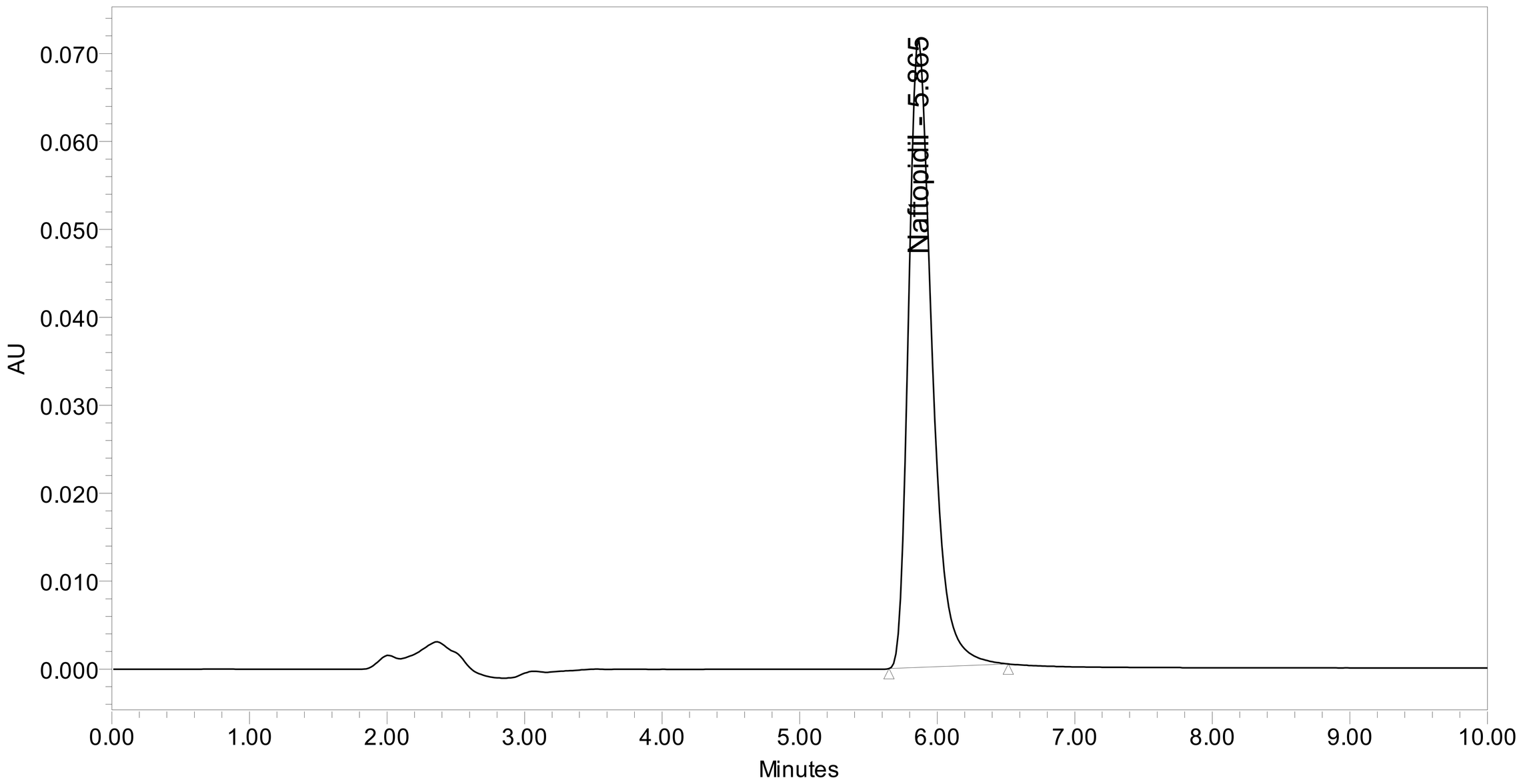
System Suitability Separation Results

	Name	RT	Resolution	Symmetry Factor	USP Plate Count	K Prime	USP Tailing
1	Naftopidil	5.869			5826.93	4.868589	1.4

System Suitability Report

SAMPLE INFORMATION

Sample Name:	Naftopidil SST_30mcg_Inj. 2	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	
Vial:	7	Acq. Method Set:	Naftopidil
Injection #:	2	Processing Method:	Naftopidil
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 282nm
Date Acquired:	4/3/2016 2:54:48 PM IST		
Date Processed:	4/3/2016 4:43:29 PM IST		



	Peak Name	RT	Area	% Area	Height	Amount	Units
1	Naftopidil	5.865	855418	100.00	71457	50.000	mcg

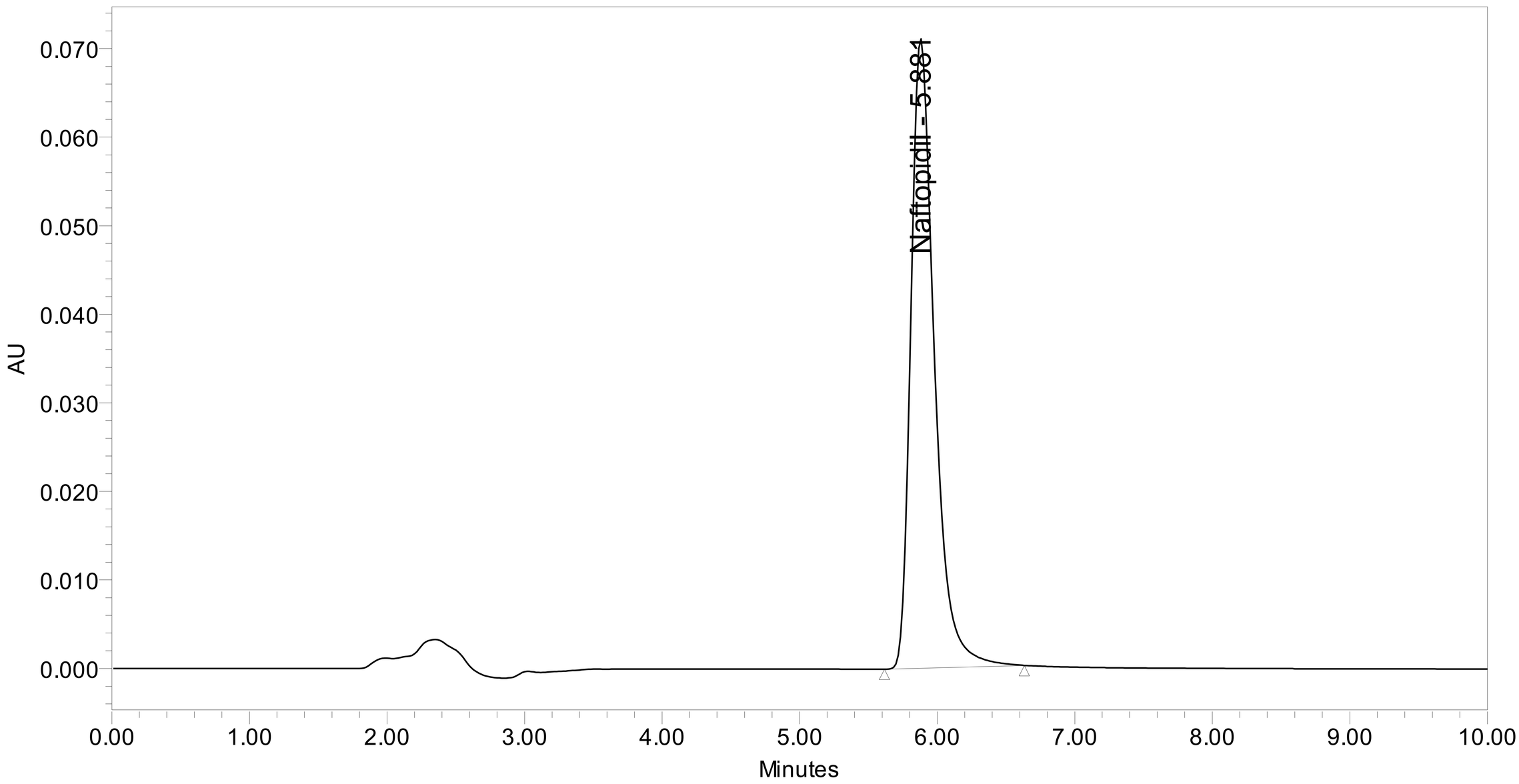
System Suitability Separation Results

	Name	RT	Resolution	Symmetry Factor	USP Plate Count	K Prime	USP Tailing
1	Naftopidil	5.865			5792.42	4.864846	1.4

System Suitability Report

SAMPLE INFORMATION

Sample Name:	Naftopidil SST_30mcg_Inj. 3	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	
Vial:	7	Acq. Method Set:	Naftopidil
Injection #:	3	Processing Method:	Naftopidil
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 282nm
Date Acquired:	4/3/2016 3:07:42 PM IST		
Date Processed:	4/3/2016 4:43:21 PM IST		



	Peak Name	RT	Area	% Area	Height	Amount	Units
1	Naftopidil	5.881	845623	100.00	71056	50.000	mcg

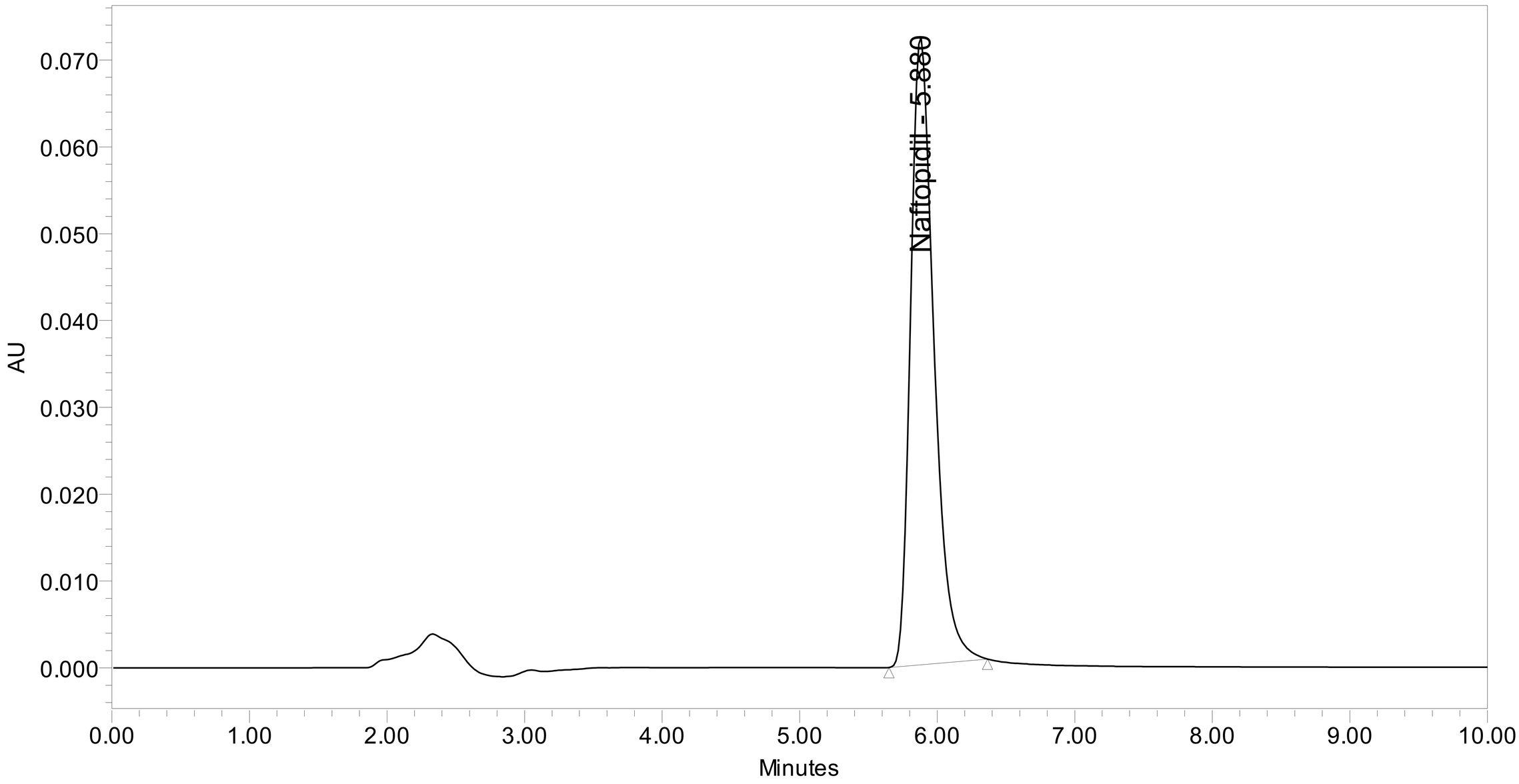
System Suitability Separation Results

	Name	RT	Resolution	Symmetry Factor	USP Plate Count	K Prime	USP Tailing
1	Naftopidil	5.881			5957.98	4.881111	1.4

System Suitability Report

SAMPLE INFORMATION

Sample Name:	Naftopidil SST_30mcg_Inj. 4	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	
Vial:	7	Acq. Method Set:	Naftopidil
Injection #:	4	Processing Method:	Naftopidil
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 282nm
Date Acquired:	4/3/2016 3:18:43 PM IST		
Date Processed:	4/3/2016 4:43:11 PM IST		



	Peak Name	RT	Area	% Area	Height	Amount	Units
1	Naftopidil	5.880	856132	100.00	72269	50.000	mcg

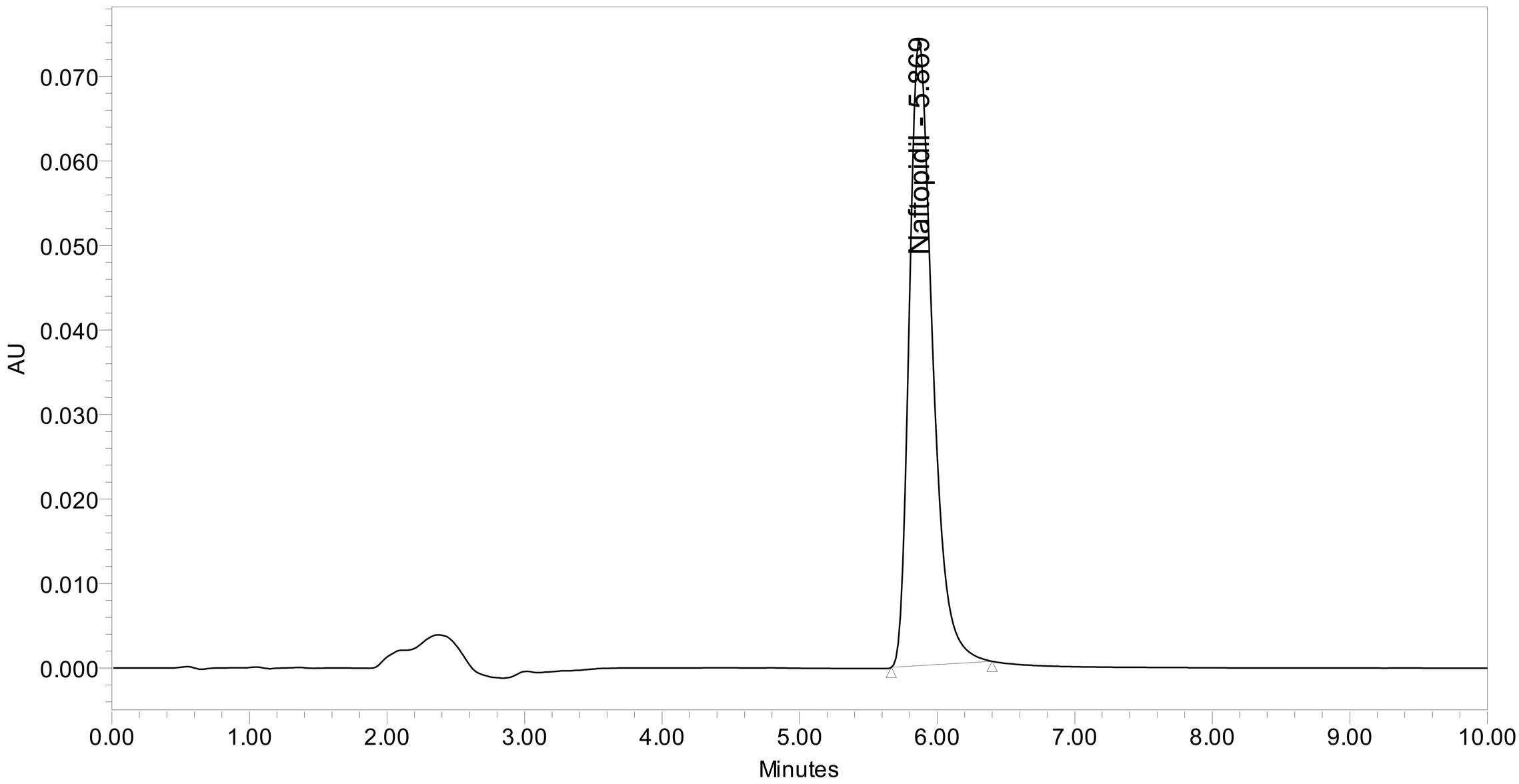
System Suitability Separation Results

	Name	RT	Resolution	Symmetry Factor	USP Plate Count	K Prime	USP Tailing
1	Naftopidil	5.880			5850.43	4.879718	1.3

System Suitability Report

SAMPLE INFORMATION

Sample Name:	Naftopidil SST_30mcg_Inj. 5	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	
Vial:	7	Acq. Method Set:	Naftopidil
Injection #:	5	Processing Method:	Naftopidil
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 282nm
Date Acquired:	4/3/2016 3:31:20 PM IST		
Date Processed:	4/3/2016 4:43:07 PM IST		



	Peak Name	RT	Area	% Area	Height	Amount	Units
1	Naftopidil	5.869	874543	100.00	74177	50.000	mcg

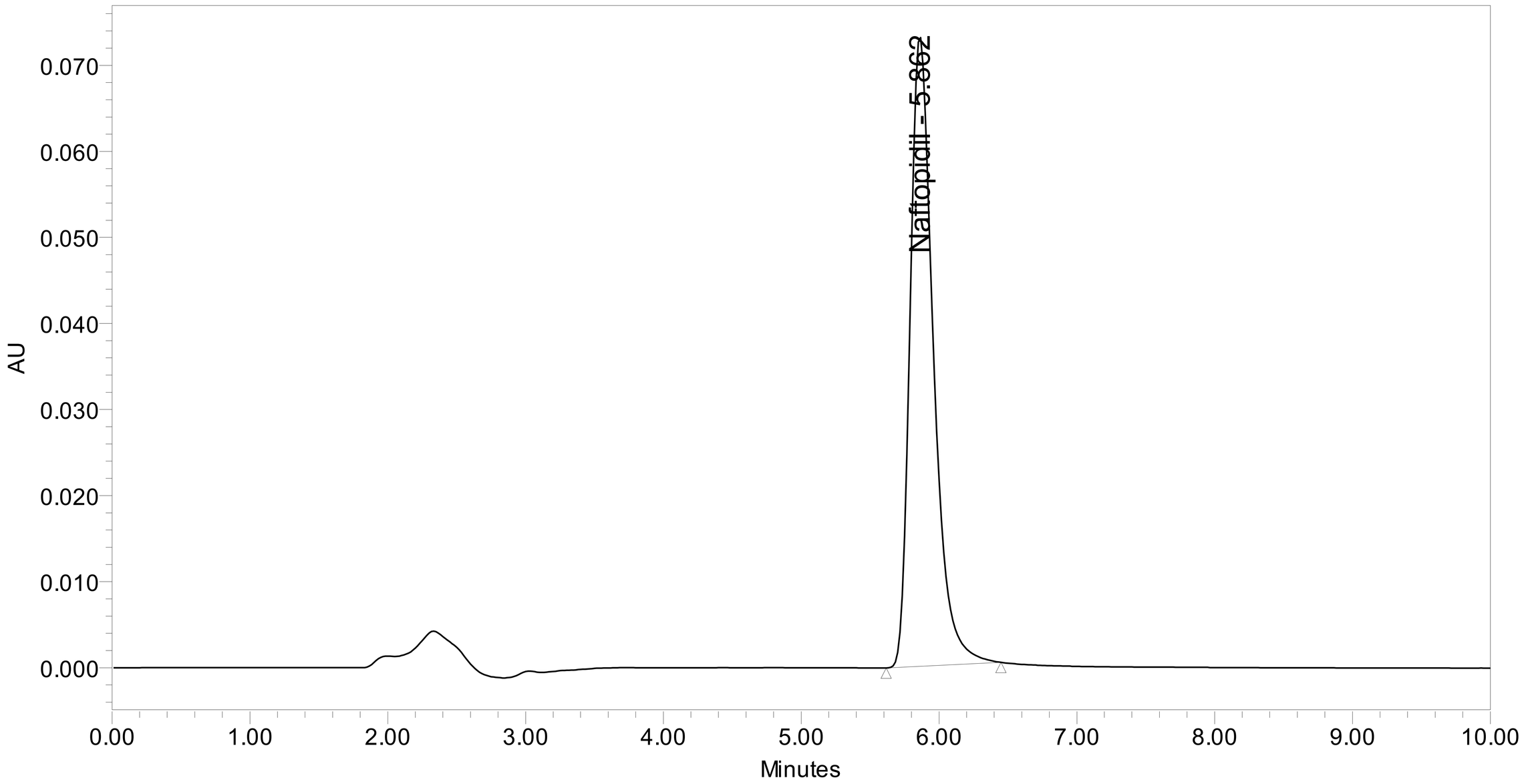
System Suitability Separation Results

	Name	RT	Resolution	Symmetry Factor	USP Plate Count	K Prime	USP Tailing
1	Naftopidil	5.869			5835.35	4.869391	1.4

System Suitability Report

SAMPLE INFORMATION

Sample Name:	Naftopidil SST_30mcg_Inj. 6	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	
Vial:	7	Acq. Method Set:	Naftopidil
Injection #:	6	Processing Method:	Naftopidil
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 282nm
Date Acquired:	4/3/2016 3:42:41 PM IST		
Date Processed:	4/3/2016 4:43:02 PM IST		



	Peak Name	RT	Area	% Area	Height	Amount	Units
1	Naftopidil	5.862	861593	100.00	73150	50.000	mcg

System Suitability Separation Results

	Name	RT	Resolution	Symmetry Factor	USP Plate Count	K Prime	USP Tailing
1	Naftopidil	5.862			6003.22	4.862005	1.4

Component Summary For Retention Time
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil SST_30mcg_Inj. 1	1	W2489 ChA	7	5.869
2	Naftopidil SST_30mcg_Inj. 2	2	W2489 ChA	7	5.865
3	Naftopidil SST_30mcg_Inj. 3	3	W2489 ChA	7	5.881
4	Naftopidil SST_30mcg_Inj. 4	4	W2489 ChA	7	5.880
5	Naftopidil SST_30mcg_Inj. 5	5	W2489 ChA	7	5.869
6	Naftopidil SST_30mcg_Inj. 6	6	W2489 ChA	7	5.862
Mean					5.871
Std. Dev.					0.008
% RSD					0.1

Component Summary For Area
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil SST_30mcg_Inj. 1	1	W2489 ChA	7	840804
2	Naftopidil SST_30mcg_Inj. 2	2	W2489 ChA	7	855418
3	Naftopidil SST_30mcg_Inj. 3	3	W2489 ChA	7	845623
4	Naftopidil SST_30mcg_Inj. 4	4	W2489 ChA	7	856132
5	Naftopidil SST_30mcg_Inj. 5	5	W2489 ChA	7	874543
6	Naftopidil SST_30mcg_Inj. 6	6	W2489 ChA	7	861593
Mean					855685
Std. Dev.					11946
% RSD					1.4

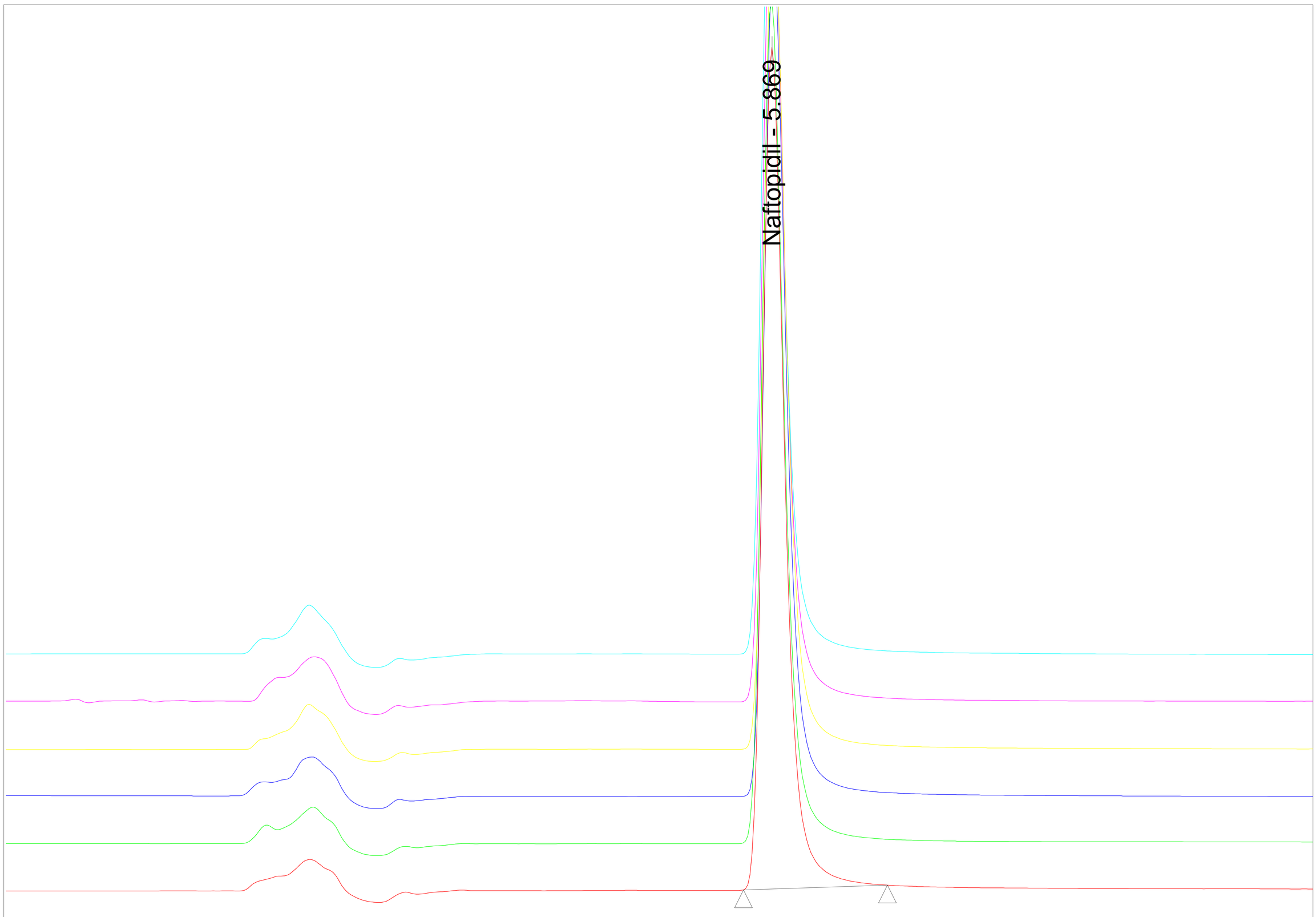
Component Summary For Amount
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil SST_30mcg_Inj. 1	1	W2489 ChA	7	50.000
2	Naftopidil SST_30mcg_Inj. 2	2	W2489 ChA	7	50.000
3	Naftopidil SST_30mcg_Inj. 3	3	W2489 ChA	7	50.000
4	Naftopidil SST_30mcg_Inj. 4	4	W2489 ChA	7	50.000
5	Naftopidil SST_30mcg_Inj. 5	5	W2489 ChA	7	50.000
6	Naftopidil SST_30mcg_Inj. 6	6	W2489 ChA	7	50.000

Component Summary For Amount
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
Mean					50.000
Std. Dev.					0.000
% RSD					0.0

Peak Summary Report



Sample Name: Naftopidil SST_30mcg_Inj. 1; Date Acquired: 4/3/2016 2:43:51 PM IST; Vial: 7; Injection: 1
Sample Name: Naftopidil SST_30mcg_Inj. 2; Date Acquired: 4/3/2016 2:54:48 PM IST; Vial: 7; Injection: 2
Sample Name: Naftopidil SST_30mcg_Inj. 3; Date Acquired: 4/3/2016 3:07:42 PM IST; Vial: 7; Injection: 3
Sample Name: Naftopidil SST_30mcg_Inj. 4; Date Acquired: 4/3/2016 3:18:43 PM IST; Vial: 7; Injection: 4
Sample Name: Naftopidil SST_30mcg_Inj. 5; Date Acquired: 4/3/2016 3:31:20 PM IST; Vial: 7; Injection: 5
Sample Name: Naftopidil SST_30mcg_Inj. 6; Date Acquired: 4/3/2016 3:42:41 PM IST; Vial: 7; Injection: 6

Peak Summary with Statistics
Name: Naftopidil

	Sample Name	Vial	Inj	Name	Retention Time (min)	Area	% Area	Height	Amount
1	Naftopidil SST_30mcg_Inj. 6	7	6	Naftopidil	5.862	861593	100.00	73150	50.0
2	Naftopidil SST_30mcg_Inj. 5	7	5	Naftopidil	5.869	874543	100.00	74177	50.0
3	Naftopidil SST_30mcg_Inj. 1	7	1	Naftopidil	5.869	840804	100.00	69794	50.0
4	Naftopidil SST_30mcg_Inj. 3	7	3	Naftopidil	5.881	845623	100.00	71056	50.0
5	Naftopidil SST_30mcg_Inj. 2	7	2	Naftopidil	5.865	855418	100.00	71457	50.0

Peak Summary with Statistics
Name: Naftopidil

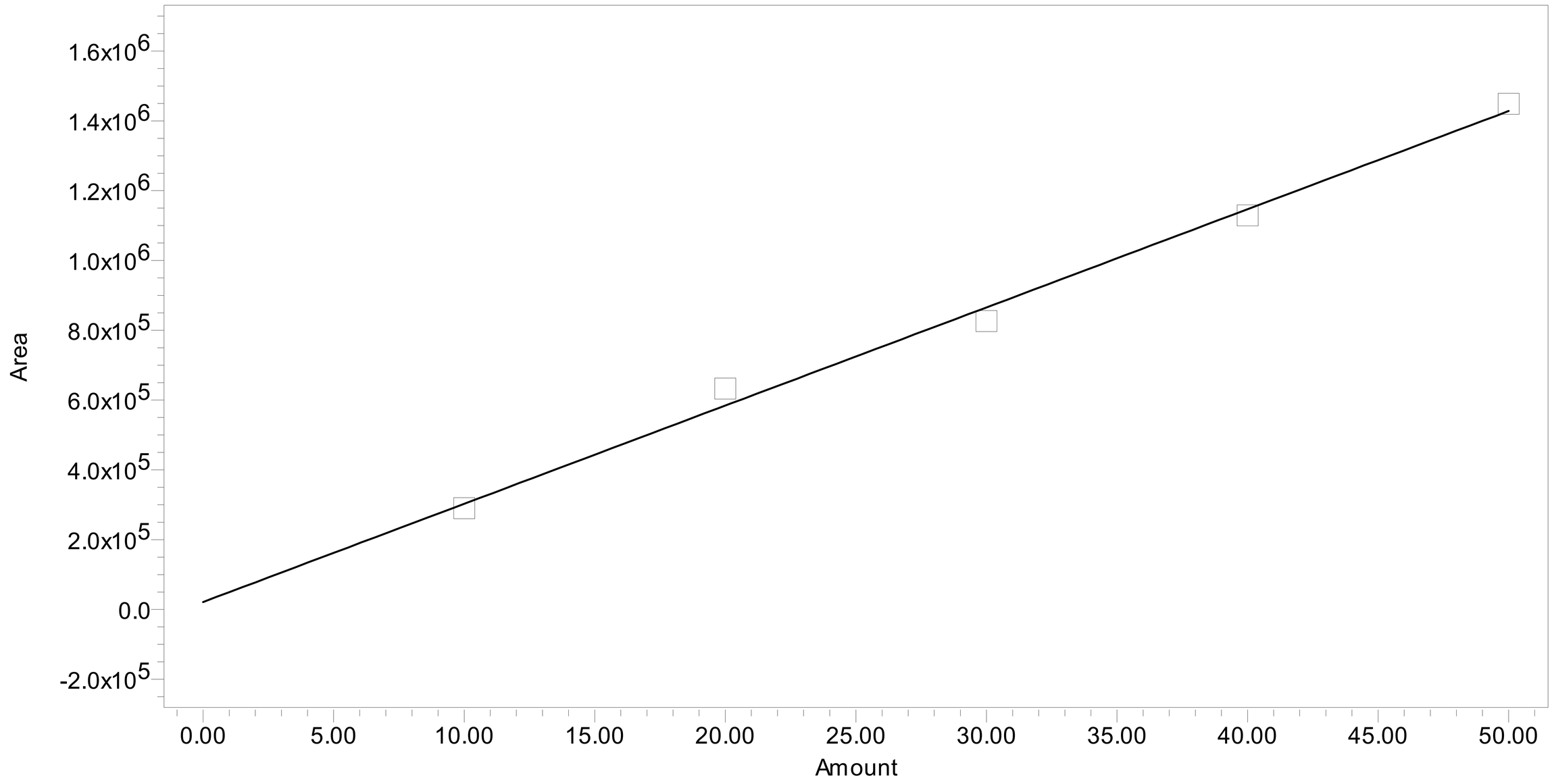
	Sample Name	Vial	Inj	Name	Retention Time (min)	Area	% Area	Height	Amount
6	Naftopidil SST_30mcg_Inj. 4	7	4	Naftopidil	5.880	856132	100.00	72269	50.0
Mean					5.871				
Std. Dev.					0.008				
% RSD					0.13				

Peak Summary with Statistics Name: Naftopidil

	Units
1	mcg
2	mcg
3	mcg
4	mcg
5	mcg
6	mcg
Mean	
Std. Dev.	
% RSD	

LC Calibration Report

Processing Method:	Naftopidil	System:	HPLC
Processing Method ID:	11497	Channel:	W2489 ChA
Calibration ID:	11549	Proc. Chnl. Descr.:	W2489 ChA 282nm
Date Calibrated:	4/3/2016 3:53:15 PM IST		



Peak Name: Naftopidil; RT: 5.524; Fit Type: Linear (1st Order); Cal Curve Id: 11550; R: 0.997015; R^2: 0.994038; Weighting: None; Equation: Y = 2.81e+004 X + 2.14e+004; Normalized Intercept/Slope: 0.025360; RSD(E): 4.596536

Peak: Naftopidil

	Sample Name	Result Id	Peak Name	Level	X Value	Response	Calc. Value	% Deviation	Manual
1	Naftopidil Lin_ 10 mcg_Inj.1	11551	Naftopidil		10.000	290185.883	9.553	-4.47	No
2	Naftopidil Lin_ 20 mcg_Inj.1	11552	Naftopidil		20.000	632622.804	21.724	8.62	No
3	Naftopidil Lin_ 30 mcg_Inj.1	11553	Naftopidil		30.000	826558.458	28.617	-4.61	No
4	Naftopidil Lin_ 40 mcg_Inj.1	11554	Naftopidil		40.000	1129330.343	39.379	-1.55	No
5	Naftopidil Lin_ 50 mcg_Inj.1	11555	Naftopidil		50.000	1448583.396	50.726	1.45	No

Peak: Naftopidil

	Ignore
1	No

	Ignore
2	No

	Ignore
3	No

	Ignore
4	No

	Ignore
5	No

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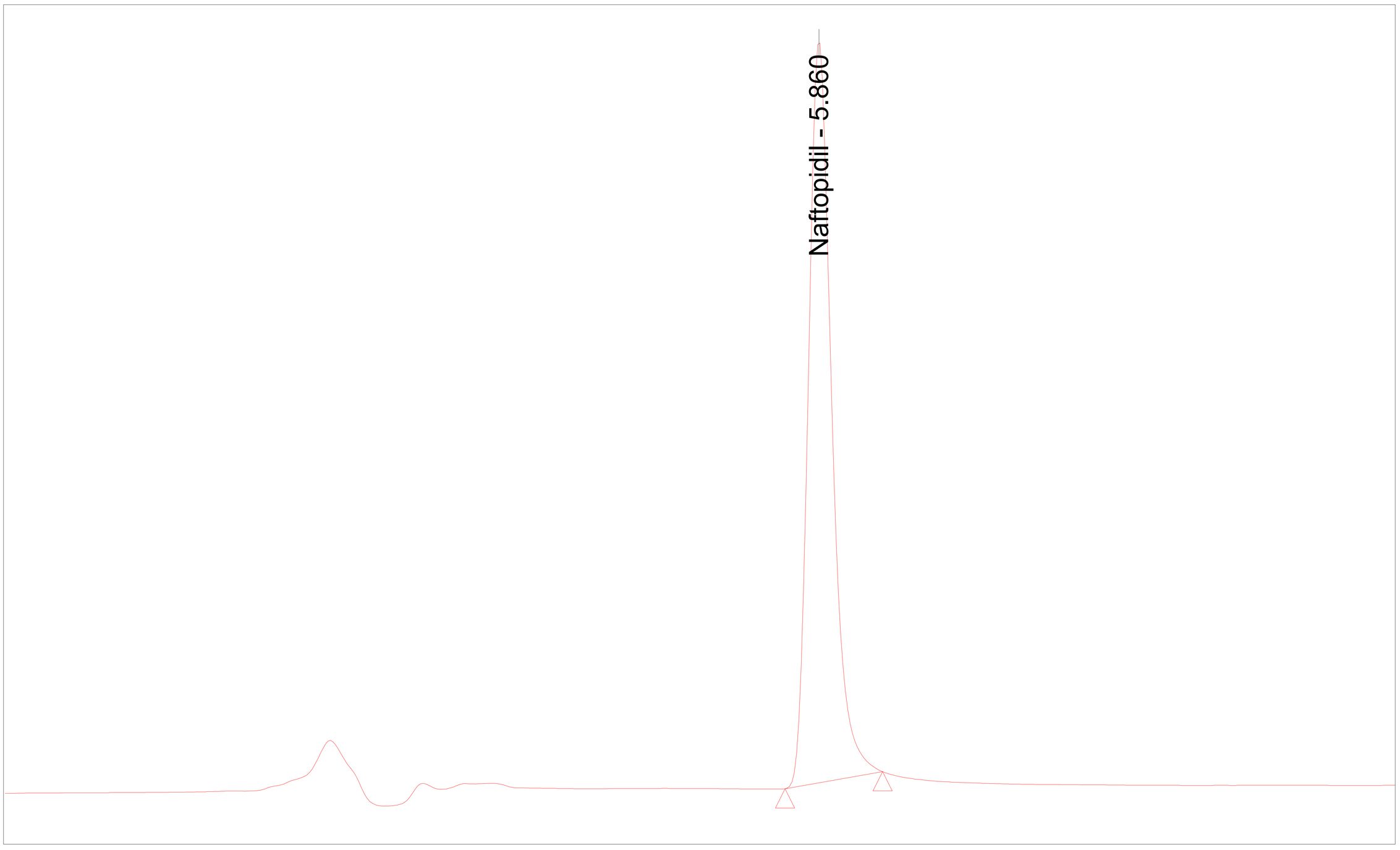
SAMPLE INFORMATION

Sample Name:	Naftopidil Lin_ 10 mcg_Inj.1	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	
Vial:	2	Acq. Method Set:	Naftopidil
Injection #:	1	Processing Method:	Naftopidil
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 282nm
Date Acquired:	4/3/2016 1:39:22 PM IST		
Date Processed:	4/3/2016 2:46:04 PM IST		

Auto-Scaled Chromatogram



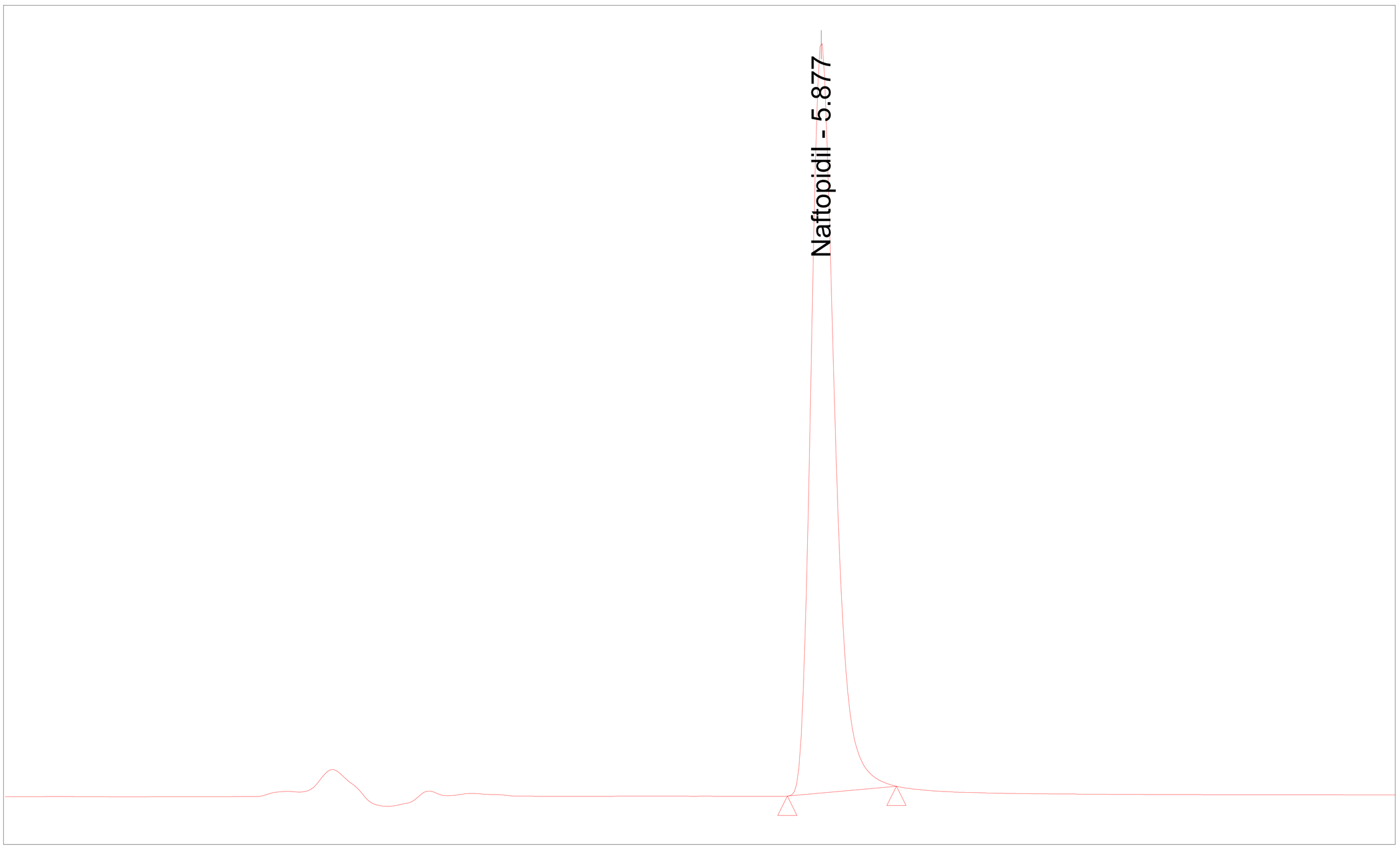
Peak Results

	Name	RT	Area	Height	Amount	Units
1	Naftopidil	5.860	290186	24949	50.000	mcg

SAMPLE INFORMATION

Sample Name:	Naftopidil Lin_ 20 mcg_Inj.1	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	
Vial:	3	Acq. Method Set:	Naftopidil
Injection #:	1	Processing Method:	Naftopidil
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 282nm
Date Acquired:	4/3/2016 1:50:35 PM IST		
Date Processed:	4/3/2016 2:46:04 PM IST		

Auto-Scaled Chromatogram



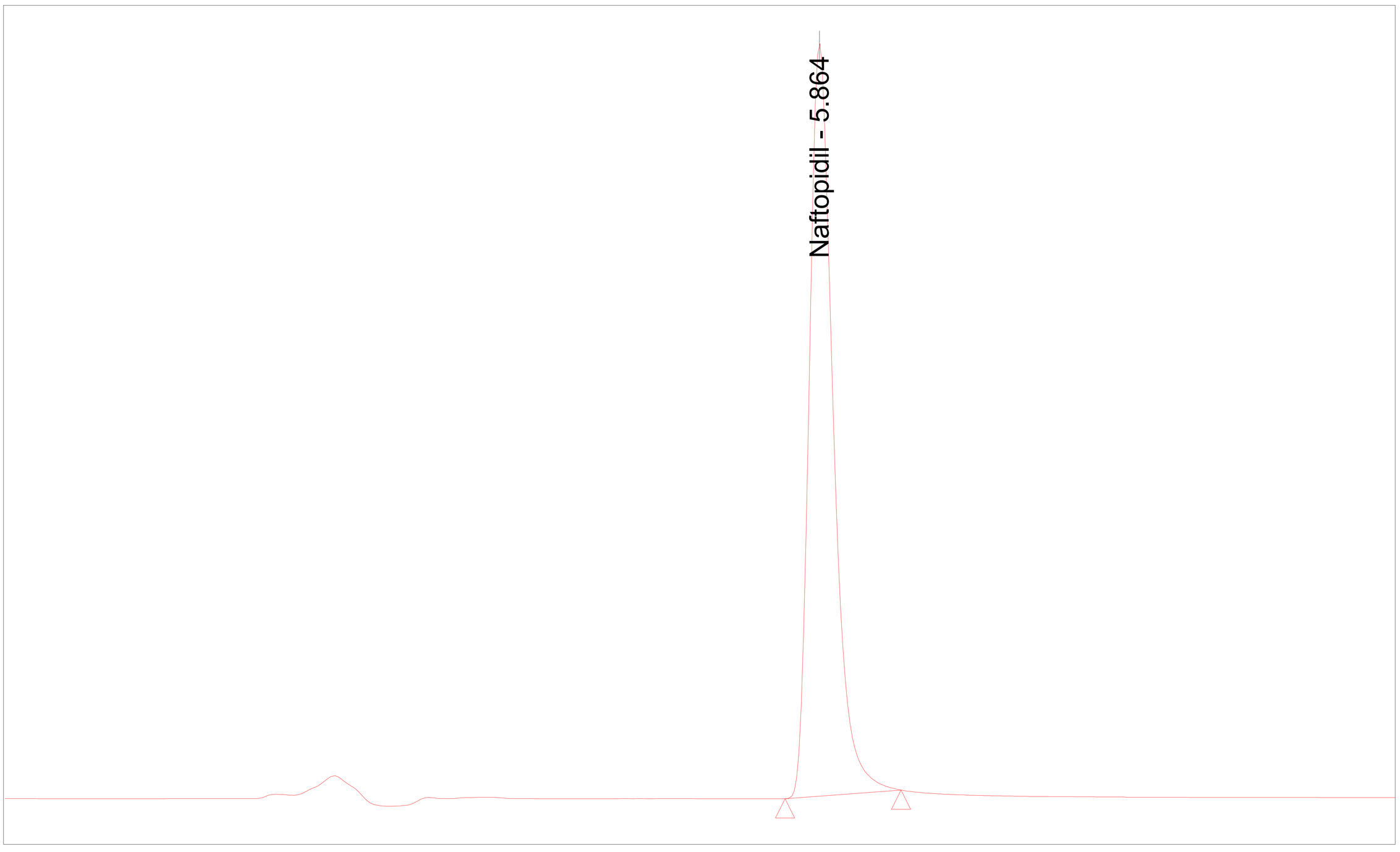
Peak Results

	Name	RT	Area	Height	Amount	Units
1	Naftopidil	5.877	632623	53260	50.000	mcg

SAMPLE INFORMATION

Sample Name:	Naftopidil Lin_ 30 mcg_Inj.1	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	
Vial:	4	Acq. Method Set:	Naftopidil
Injection #:	1	Processing Method:	Naftopidil
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 282nm
Date Acquired:	4/3/2016 2:02:10 PM IST		
Date Processed:	4/3/2016 2:46:04 PM IST		

Auto-Scaled Chromatogram



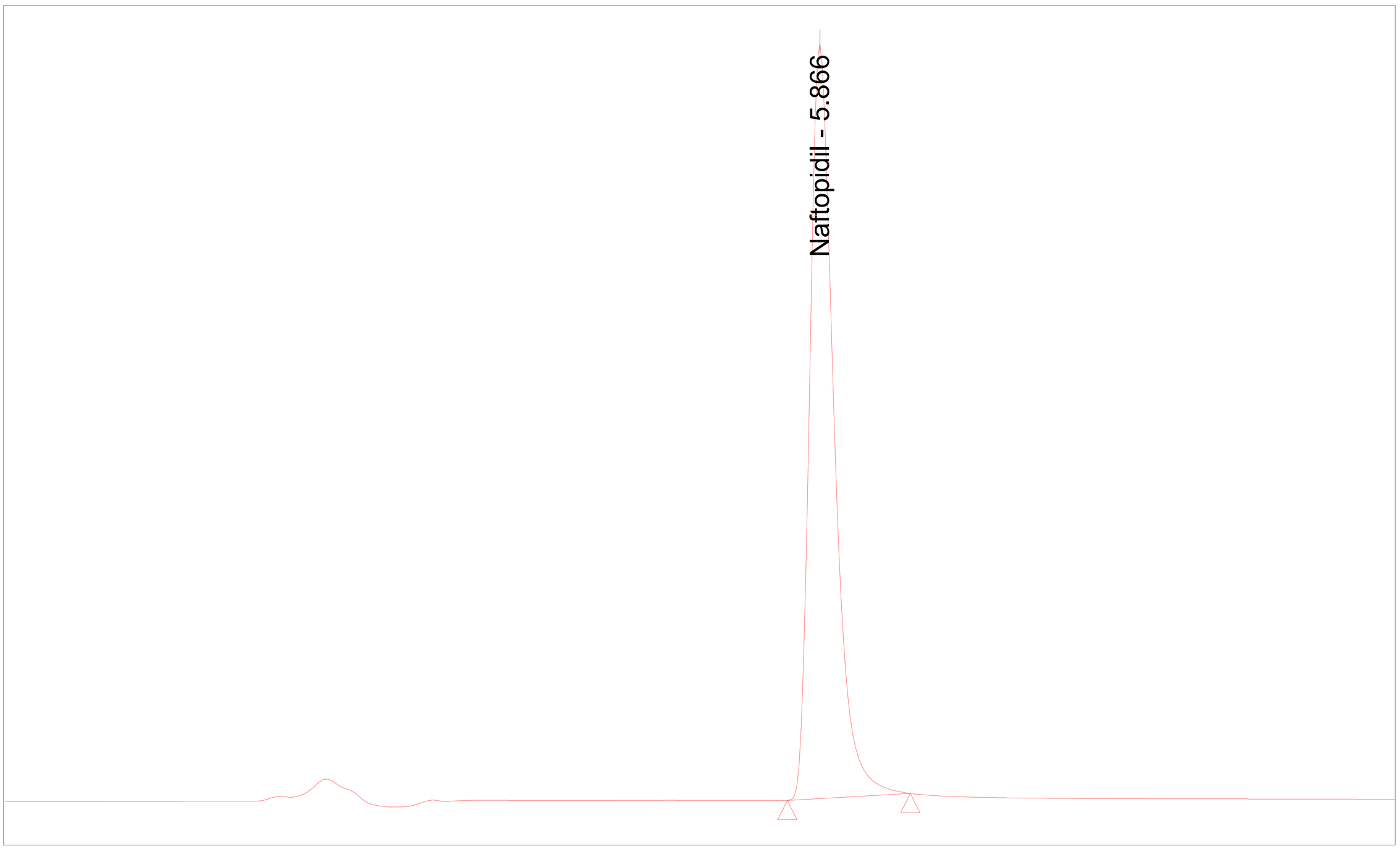
Peak Results

	Name	RT	Area	Height	Amount	Units
1	Naftopidil	5.864	826558	68919	50.000	mcg

SAMPLE INFORMATION

Sample Name:	Naftopidil Lin_ 40 mcg_Inj.1	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	
Vial:	5	Acq. Method Set:	Naftopidil
Injection #:	1	Processing Method:	Naftopidil
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 282nm
Date Acquired:	4/3/2016 2:14:10 PM IST		
Date Processed:	4/3/2016 2:46:04 PM IST		

Auto-Scaled Chromatogram



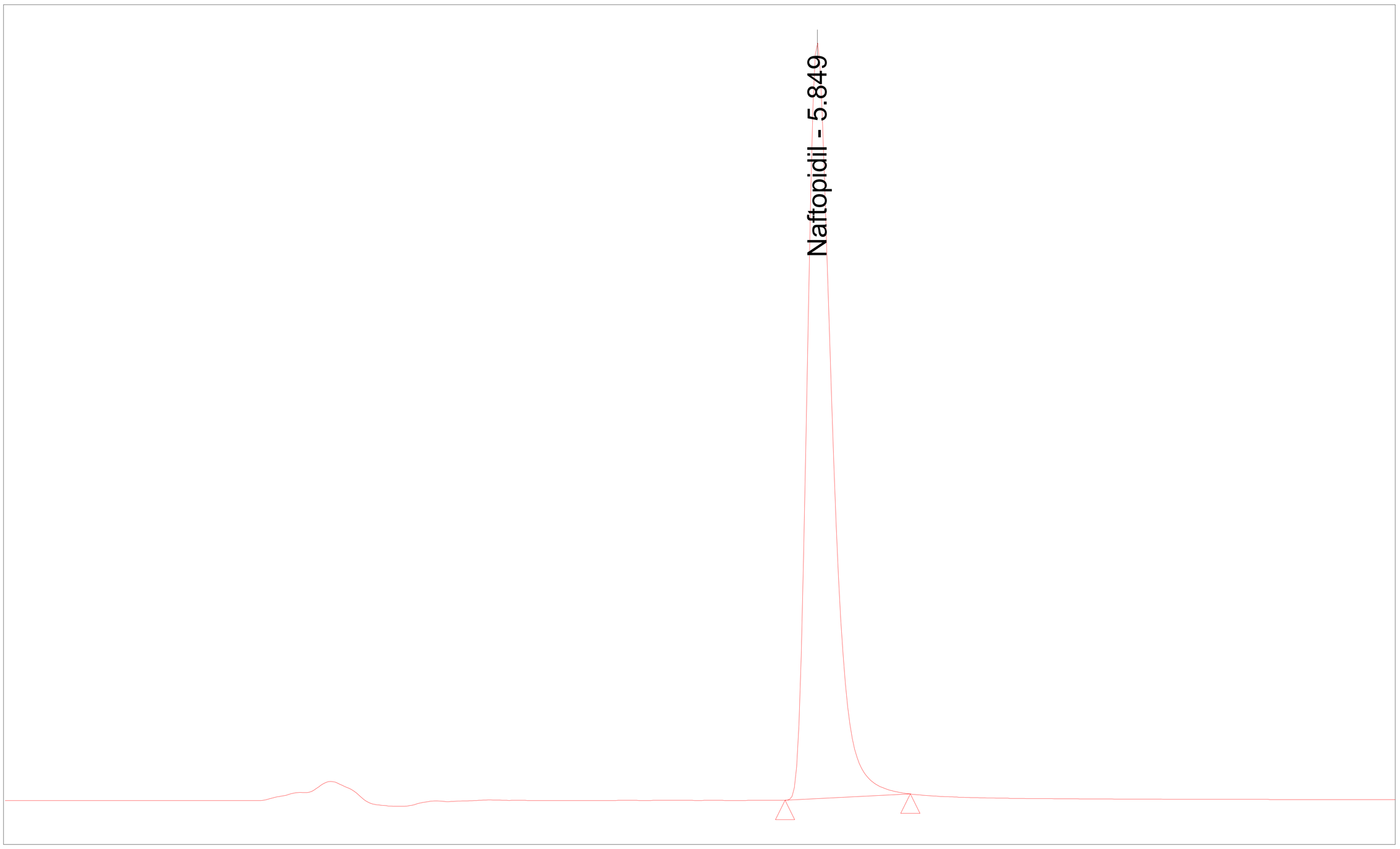
Peak Results

	Name	RT	Area	Height	Amount	Units
1	Naftopidil	5.866	1129330	93010	50.000	mcg

SAMPLE INFORMATION

Sample Name:	Naftopidil Lin_ 50 mcg_Inj.1	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	
Vial:	6	Acq. Method Set:	Naftopidil
Injection #:	1	Processing Method:	Naftopidil
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 282nm
Date Acquired:	4/3/2016 2:25:20 PM IST		
Date Processed:	4/3/2016 2:46:04 PM IST		

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Height	Amount	Units
1	Naftopidil	5.849	1448583	116438	50.000	mcg

Component Summary For Retention Time
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil Intra_Preci_90%_Inj 1	1	W2489 ChA	2	5.924
2	Naftopidil Intra_Preci_90%_Inj 2	2	W2489 ChA	2	5.936
3	Naftopidil Intra_Preci_90%_Inj 3	3	W2489 ChA	2	5.942
Mean					5.934
Std. Dev.					0.009
% RSD					0.1

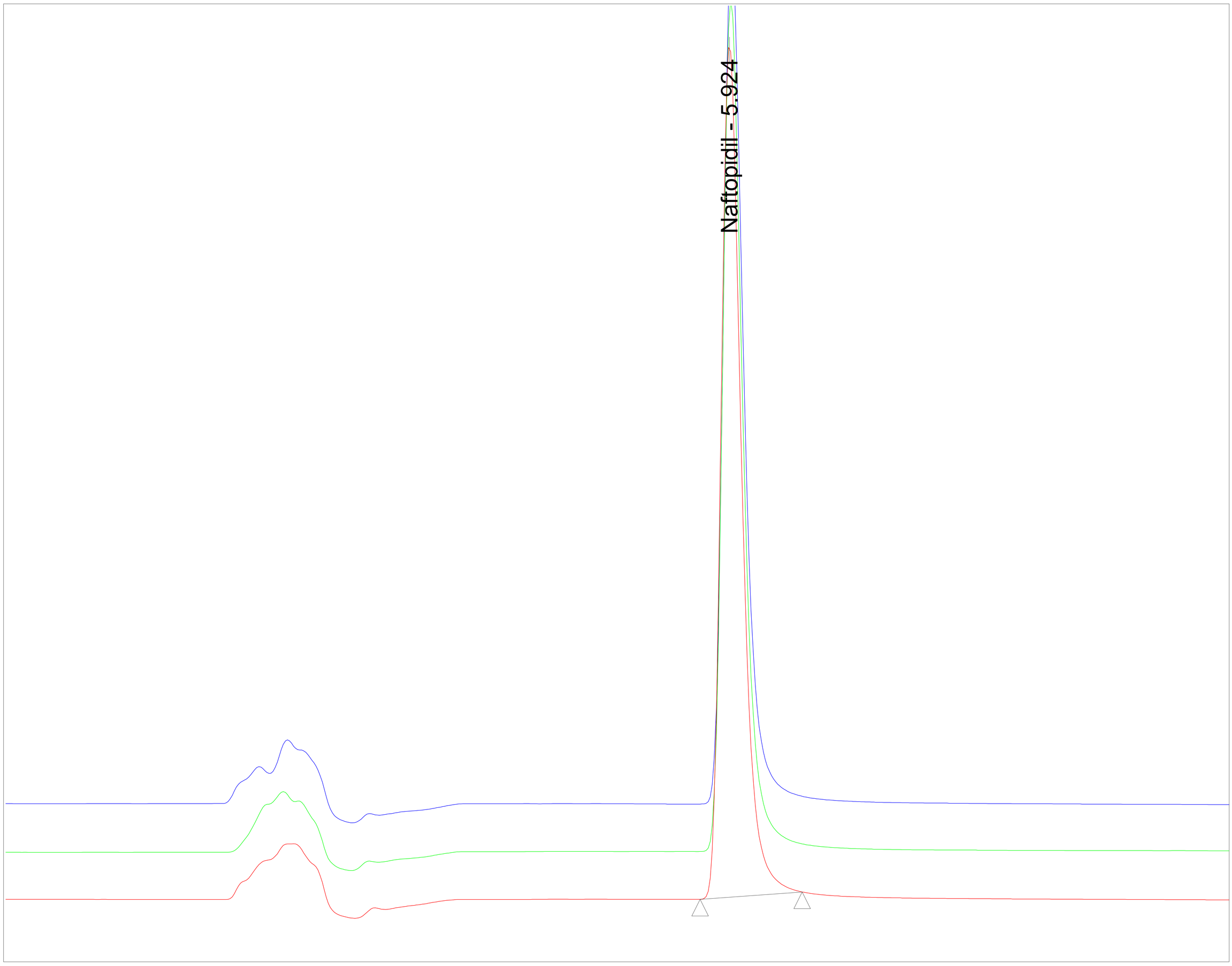
Component Summary For Area
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil Intra_Preci_90%_Inj 1	1	W2489 ChA	2	824459
2	Naftopidil Intra_Preci_90%_Inj 2	2	W2489 ChA	2	803106
3	Naftopidil Intra_Preci_90%_Inj 3	3	W2489 ChA	2	818985
Mean					815517
Std. Dev.					11091
% RSD					1.4

Component Summary For Amount
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil Intra_Preci_90%_Inj 1	1	W2489 ChA	2	50.000
2	Naftopidil Intra_Preci_90%_Inj 2	2	W2489 ChA	2	50.000
3	Naftopidil Intra_Preci_90%_Inj 3	3	W2489 ChA	2	50.000
Mean					50.000
Std. Dev.					0.000
% RSD					0.0

Peak Summary Report



Sample Name: Naftopidil Intra_Preci_90%_Inj 1; Date Acquired: 4/4/2016 2:08:51 PM IST; Vial: 2; Injection: 1
Sample Name: Naftopidil Intra_Preci_90%_Inj 2; Date Acquired: 4/4/2016 2:20:04 PM IST; Vial: 2; Injection: 2
Sample Name: Naftopidil Intra_Preci_90%_Inj 3; Date Acquired: 4/4/2016 2:31:13 PM IST; Vial: 2; Injection: 3

Peak Summary with Statistics
Name: Naftopidil

	Sample Name	Vial	Inj	Name	Retention Time (min)	Area	% Area	Height	Amount
1	Naftopidil Intra_Preci_90%_Inj 1	2	1	Naftopidil	5.924	824459	100.00	70419	50.0
2	Naftopidil Intra_Preci_90%_Inj 3	2	3	Naftopidil	5.942	818985	100.00	70159	50.0
3	Naftopidil Intra_Preci_90%_Inj 2	2	2	Naftopidil	5.936	803106	100.00	69097	50.0
Mean					5.934				
Std. Dev.					0.009				

Peak Summary with Statistics
Name: Naftopidil

	Sample Name	Vial	Inj	Name	Retention Time (min)	Area	% Area	Height	Amount
% RSD					0.15				

Peak Summary with Statistics Name: Naftopidil

	Units
1	mcg
2	mcg
3	mcg
Mean	
Std. Dev.	
% RSD	

Component Summary For Retention Time
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil Intra_Prec_100%_Inj 1	1	W2489 ChA	3	5.921
2	Naftopidil Intra_Prec_100%_Inj 2	2	W2489 ChA	3	5.939
3	Naftopidil Intra_Prec_100%_Inj 3	3	W2489 ChA	3	5.952
Mean					5.937
Std. Dev.					0.016
% RSD					0.3

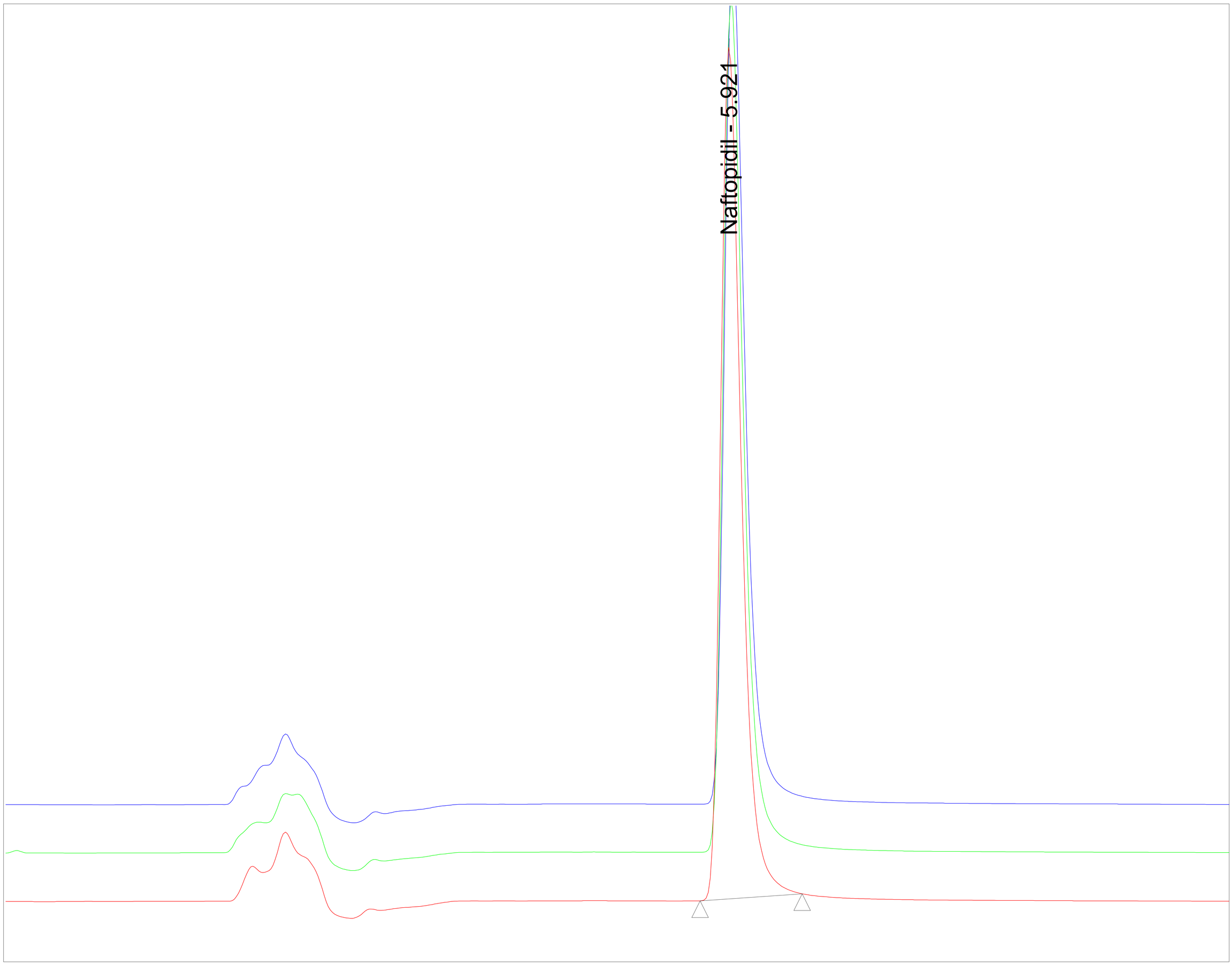
Component Summary For Area
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil Intra_Prec_100%_Inj 1	1	W2489 ChA	3	885643
2	Naftopidil Intra_Prec_100%_Inj 2	2	W2489 ChA	3	886301
3	Naftopidil Intra_Prec_100%_Inj 3	3	W2489 ChA	3	869717
Mean					880554
Std. Dev.					9391
% RSD					1.1

Component Summary For Amount
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil Intra_Prec_100%_Inj 1	1	W2489 ChA	3	50.000
2	Naftopidil Intra_Prec_100%_Inj 2	2	W2489 ChA	3	50.000
3	Naftopidil Intra_Prec_100%_Inj 3	3	W2489 ChA	3	50.000
Mean					50.000
Std. Dev.					0.000
% RSD					0.0

Peak Summary Report



Sample Name: Naftopidil Intra_Prec_100%_Inj 1; Date Acquired: 4/4/2016 2:42:34 PM IST; Vial: 3; Injection: 1
Sample Name: Naftopidil Intra_Prec_100%_Inj 2; Date Acquired: 4/4/2016 2:53:37 PM IST; Vial: 3; Injection: 2
Sample Name: Naftopidil Intra_Prec_100%_Inj 3; Date Acquired: 4/4/2016 3:04:32 PM IST; Vial: 3; Injection: 3

Peak Summary with Statistics
Name: Naftopidil

	Sample Name	Vial	Inj	Name	Retention Time (min)	Area	% Area	Height
1	Naftopidil Intra_Prec_100%_Inj 1	3	1	Naftopidil	5.921	885643	100.00	75520
2	Naftopidil Intra_Prec_100%_Inj 3	3	3	Naftopidil	5.952	869717	100.00	74476
3	Naftopidil Intra_Prec_100%_Inj 2	3	2	Naftopidil	5.939	886301	100.00	75865
Mean					5.937			
Std. Dev.					0.016			

Peak Summary with Statistics
Name: Naftopidil

	Sample Name	Vial	Inj	Name	Retention Time (min)	Area	% Area	Height
% RSD					0.26			

Peak Summary with Statistics
Name: Naftopidil

	Amount	Units
1	50.0	mcg
2	50.0	mcg
3	50.0	mcg
Mean		
Std. Dev.		
% RSD		

Component Summary For Retention Time
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil Intra_Prec_110%_Inj 1	1	W2489 ChA	4	5.933
2	Naftopidil Intra_Prec_110%_Inj 2	1	W2489 ChA	4	5.950
3	Naftopidil Intra_Prec_110%_Inj 3	3	W2489 ChA	4	5.933
Mean					5.938
Std. Dev.					0.010
% RSD					0.2

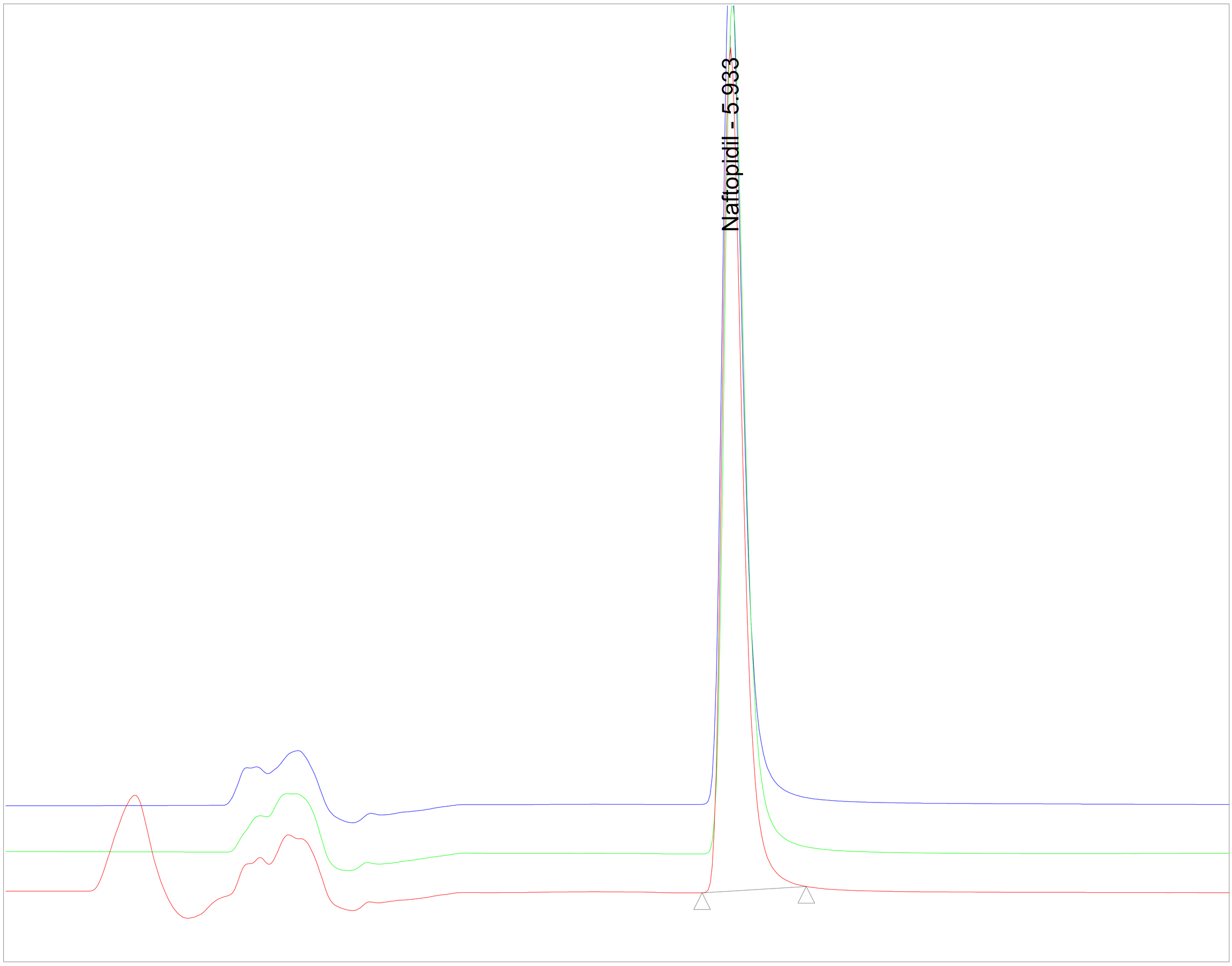
Component Summary For Area
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil Intra_Prec_110%_Inj 1	1	W2489 ChA	4	995227
2	Naftopidil Intra_Prec_110%_Inj 2	1	W2489 ChA	4	984023
3	Naftopidil Intra_Prec_110%_Inj 3	3	W2489 ChA	4	1000983
Mean					993411
Std. Dev.					8624
% RSD					0.9

Component Summary For Amount
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil Intra_Prec_110%_Inj 1	1	W2489 ChA	4	50.000
2	Naftopidil Intra_Prec_110%_Inj 2	1	W2489 ChA	4	50.000
3	Naftopidil Intra_Prec_110%_Inj 3	3	W2489 ChA	4	50.000
Mean					50.000
Std. Dev.					0.000
% RSD					0.0

Peak Summary Report



Sample Name: Naftopidil Intra_Prec_110%_Inj 1; Date Acquired: 4/4/2016 3:17:05 PM IST; Vial: 4; Injection: 1
Sample Name: Naftopidil Intra_Prec_110%_Inj 2; Date Acquired: 4/4/2016 3:28:57 PM IST; Vial: 4; Injection: 1
Sample Name: Naftopidil Intra_Prec_110%_Inj 3; Date Acquired: 4/4/2016 3:38:41 PM IST; Vial: 4; Injection: 3

Peak Summary with Statistics
Name: Naftopidil

	Sample Name	Vial	Inj	Name	Retention Time (min)	Area	% Area	Height
1	Naftopidil Intra_Prec_110%_Inj 1	4	1	Naftopidil	5.933	995227	100.00	84253
2	Naftopidil Intra_Prec_110%_Inj 3	4	3	Naftopidil	5.933	1000983	100.00	84257
3	Naftopidil Intra_Prec_110%_Inj 2	4	1	Naftopidil	5.950	984023	100.00	82270
Mean					5.938			
Std. Dev.					0.010			

Peak Summary with Statistics
Name: Naftopidil

	Sample Name	Vial	Inj	Name	Retention Time (min)	Area	% Area	Height
% RSD					0.16			

Peak Summary with Statistics
Name: Naftopidil

	Amount	Units
1	50.0	mcg
2	50.0	mcg
3	50.0	mcg
Mean		
Std. Dev.		
% RSD		

Component Summary For Retention Time
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil Inter day prec Std 01	1	W2489 ChA	2	5.960
2	Naftopidil Inter day prec Std 02	2	W2489 ChA	2	5.970
3	Naftopidil Inter day prec Std 03	3	W2489 ChA	2	5.973
Mean					5.968
Std. Dev.					0.007
% RSD					0.1

Component Summary For Area
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil Inter day prec Std 01	1	W2489 ChA	2	959771
2	Naftopidil Inter day prec Std 02	2	W2489 ChA	2	949007
3	Naftopidil Inter day prec Std 03	3	W2489 ChA	2	961097
Mean					956625
Std. Dev.					6630
% RSD					0.7

Component Summary For Amount
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil Inter day prec Std 01	1	W2489 ChA	2	50.000
2	Naftopidil Inter day prec Std 02	2	W2489 ChA	2	50.000
3	Naftopidil Inter day prec Std 03	3	W2489 ChA	2	50.000
Mean					50.000
Std. Dev.					0.000
% RSD					0.0

Component Summary For Retention Time
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Inter day prec day_3 Std 01	1	W2489 ChA	2	6.036
2	Inter day prec day_3 Std 02	4	W2489 ChA	2	6.029
3	Inter day prec day_3 Std 03	3	W2489 ChA	2	6.034
Mean					6.033
Std. Dev.					0.003
% RSD					0.1

Component Summary For Area
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Inter day prec day_3 Std 01	1	W2489 ChA	2	933612
2	Inter day prec day_3 Std 02	4	W2489 ChA	2	938969
3	Inter day prec day_3 Std 03	3	W2489 ChA	2	930966
Mean					934516
Std. Dev.					4077
% RSD					0.4

Component Summary For Amount
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Inter day prec day_3 Std 01	1	W2489 ChA	2	50.000
2	Inter day prec day_3 Std 02	4	W2489 ChA	2	50.000
3	Inter day prec day_3 Std 03	3	W2489 ChA	2	50.000
Mean					50.000
Std. Dev.					0.000
% RSD					0.0

Component Summary For Retention Time
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil Inter day prec Std 01	1	W2489 ChA	2	5.960
2	Naftopidil Inter day prec Std 02	2	W2489 ChA	2	5.970
3	Naftopidil Inter day prec Std 03	3	W2489 ChA	2	5.973
Mean					5.968
Std. Dev.					0.007
% RSD					0.1

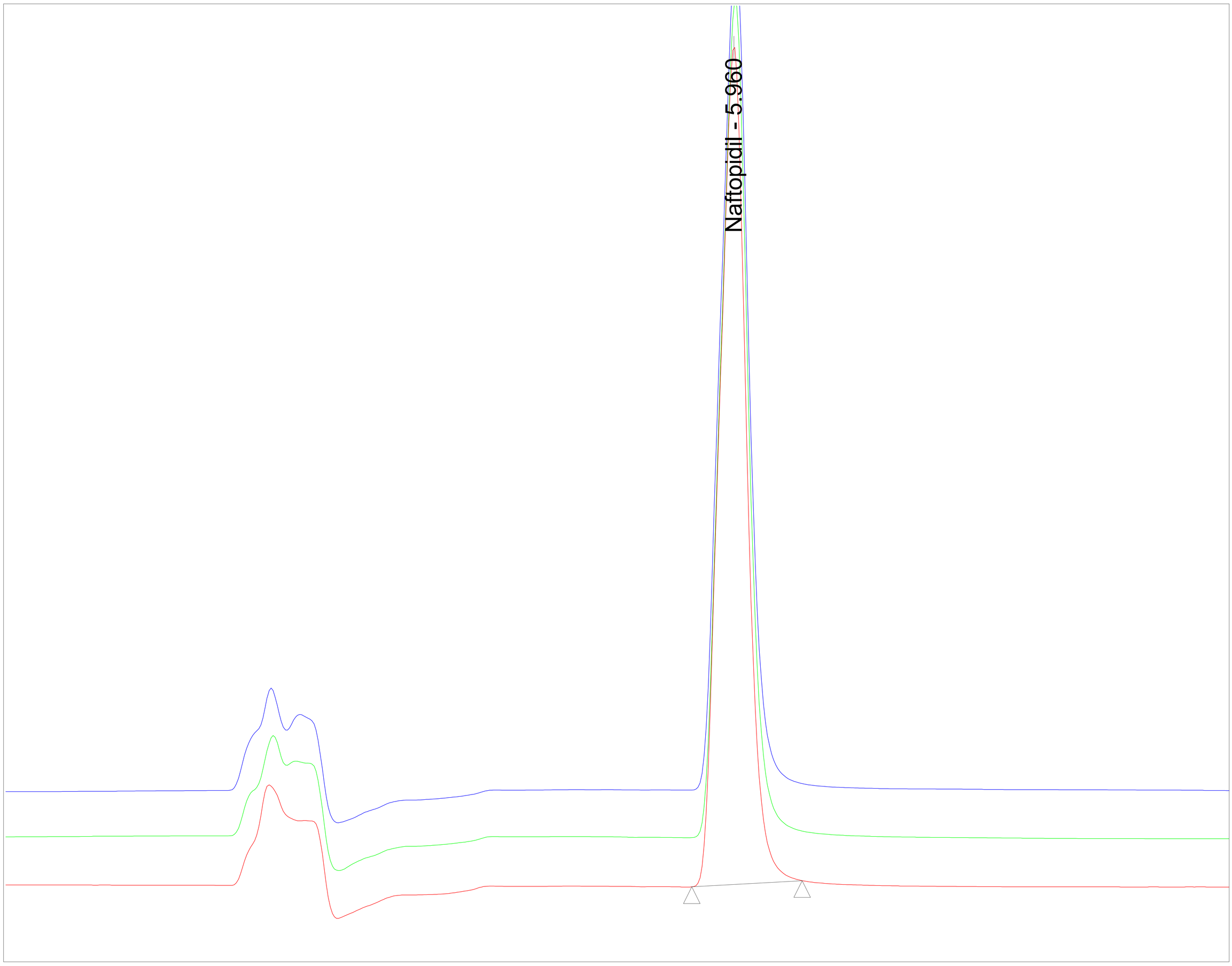
Component Summary For Area
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil Inter day prec Std 01	1	W2489 ChA	2	959771
2	Naftopidil Inter day prec Std 02	2	W2489 ChA	2	949007
3	Naftopidil Inter day prec Std 03	3	W2489 ChA	2	961097
Mean					956625
Std. Dev.					6630
% RSD					0.7

Component Summary For Amount
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil Inter day prec Std 01	1	W2489 ChA	2	50.000
2	Naftopidil Inter day prec Std 02	2	W2489 ChA	2	50.000
3	Naftopidil Inter day prec Std 03	3	W2489 ChA	2	50.000
Mean					50.000
Std. Dev.					0.000
% RSD					0.0

Peak Summary Report



Sample Name: Naftopidil Inter day prec Std 01; Date Acquired: 5/28/2016 12:52:19 PM IST; Vial: 2; Injection: 1
Sample Name: Naftopidil Inter day prec Std 02; Date Acquired: 5/28/2016 1:03:53 PM IST; Vial: 2; Injection: 2
Sample Name: Naftopidil Inter day prec Std 03; Date Acquired: 5/28/2016 1:15:31 PM IST; Vial: 2; Injection: 3

Peak Summary with Statistics
Name: Naftopidil

	Sample Name	Vial	Inj	Name	Retention Time (min)	Area	% Area	Height	Amount
1	Naftopidil Inter day prec Std 03	2	3	Naftopidil	5.973	961097	100.00	60586	50.0
2	Naftopidil Inter day prec Std 01	2	1	Naftopidil	5.960	959771	100.00	62320	50.0
3	Naftopidil Inter day prec Std 02	2	2	Naftopidil	5.970	949007	100.00	60714	50.0
Mean					5.968				
Std. Dev.					0.007				

Peak Summary with Statistics Name: Naftopidil

	Sample Name	Vial	Inj	Name	Retention Time (min)	Area	% Area	Height	Amount
% RSD					0.12				

Peak Summary with Statistics Name: Naftopidil

	Units
1	mcg
2	mcg
3	mcg
Mean	
Std. Dev.	
% RSD	

Component Summary For Retention Time
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil Accura_ Std 01	1	W2489 ChA	2	6.173
2	Naftopidil Accuracy_ Std 02	2	W2489 ChA	2	6.162
3	Naftopidil Accuracy_ Std 03	3	W2489 ChA	2	6.198
4	Naftopidil Accuracy_ Std 04	1	W2489 ChA	2	5.944
5	Naftopidil Accuracy_ Std 05	2	W2489 ChA	2	5.937
Mean					6.083
Std. Dev.					0.130
% RSD					2.1

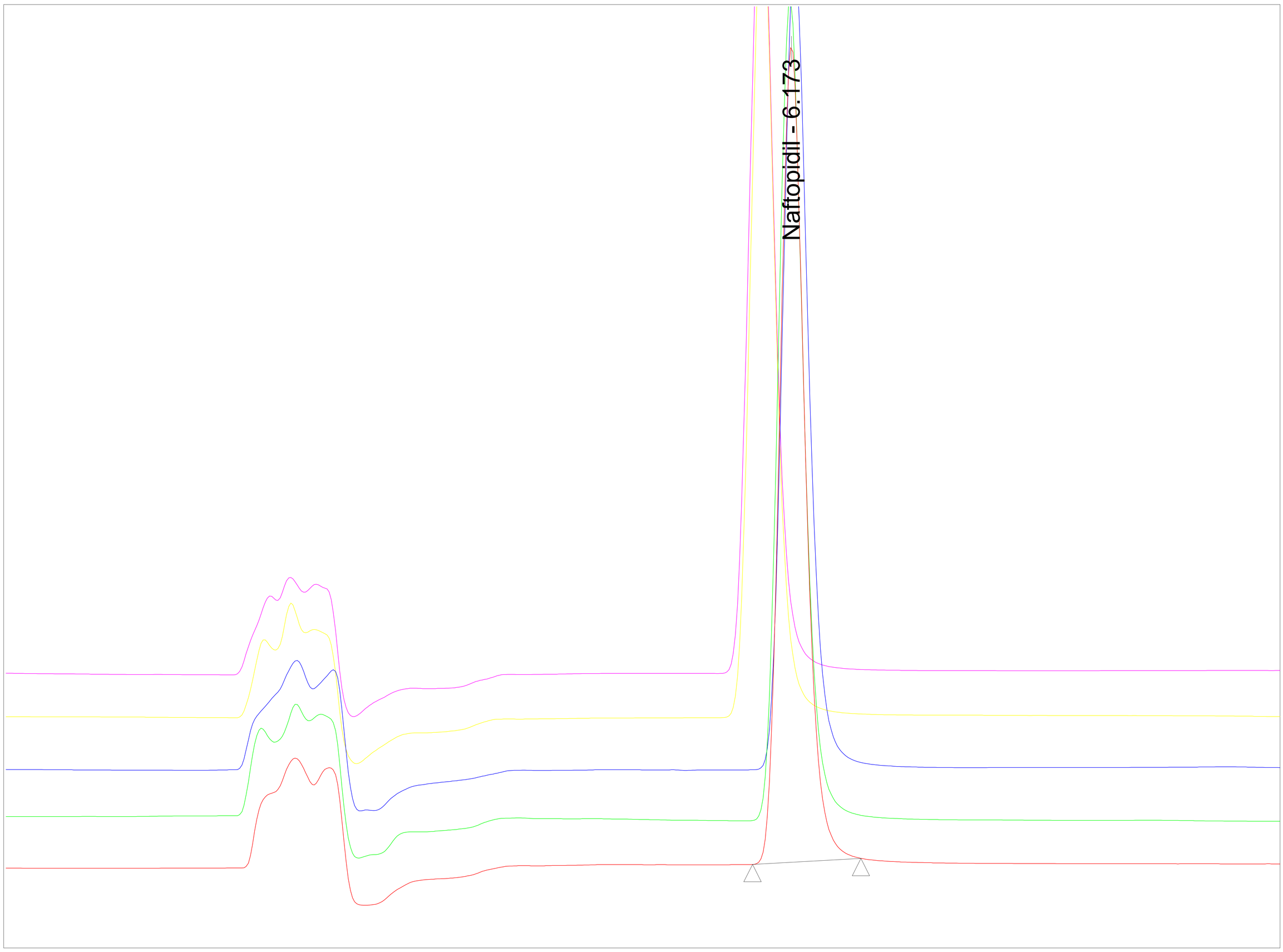
Component Summary For Area
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil Accura_ Std 01	1	W2489 ChA	2	950478
2	Naftopidil Accuracy_ Std 02	2	W2489 ChA	2	959209
3	Naftopidil Accuracy_ Std 03	3	W2489 ChA	2	969944
4	Naftopidil Accuracy_ Std 04	1	W2489 ChA	2	931247
5	Naftopidil Accuracy_ Std 05	2	W2489 ChA	2	931153
Mean					948406
Std. Dev.					17154
% RSD					1.8

Component Summary For Amount
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil Accura_ Std 01	1	W2489 ChA	2	50.000
2	Naftopidil Accuracy_ Std 02	2	W2489 ChA	2	50.000
3	Naftopidil Accuracy_ Std 03	3	W2489 ChA	2	50.000
4	Naftopidil Accuracy_ Std 04	1	W2489 ChA	2	50.000
5	Naftopidil Accuracy_ Std 05	2	W2489 ChA	2	50.000
Mean					50.000
Std. Dev.					0.000
% RSD					0.0

Peak Summary Report



Sample Name: Naftopidil Accura_ Std 01; Date Acquired: 4/11/2016 11:25:07 AM IST; Vial: 2; Injection: 1
Sample Name: Naftopidil Accuracy_ Std 02; Date Acquired: 4/11/2016 11:36:38 AM IST; Vial: 2; Injection: 2
Sample Name: Naftopidil Accuracy_ Std 03; Date Acquired: 4/11/2016 11:47:39 AM IST; Vial: 2; Injection: 3
Sample Name: Naftopidil Accuracy_ Std 04; Date Acquired: 4/11/2016 12:12:11 PM IST; Vial: 2; Injection: 1
Sample Name: Naftopidil Accuracy_ Std 05; Date Acquired: 4/11/2016 12:23:13 PM IST; Vial: 2; Injection: 2

Peak Summary with Statistics
Name: Naftopidil

	Sample Name	Vial	Inj	Name	Retention Time (min)	Area	% Area	Height	Amount
1	Naftopidil Accura_ Std 01	2	1	Naftopidil	6.173	950478	100.00	69471	50.0
2	Naftopidil Accuracy_ Std 02	2	2	Naftopidil	6.162	959209	100.00	69105	50.0
3	Naftopidil Accuracy_ Std 05	2	2	Naftopidil	5.937	931153	100.00	67386	50.0
4	Naftopidil Accuracy_ Std 04	2	1	Naftopidil	5.944	931247	100.00	68980	50.0
5	Naftopidil Accuracy_ Std 03	2	3	Naftopidil	6.198	969944	100.00	69941	50.0

Peak Summary with Statistics
Name: Naftopidil

	Sample Name	Vial	Inj	Name	Retention Time (min)	Area	% Area	Height	Amount
Mean					6.083				
Std. Dev.					0.130				
% RSD					2.14				

Peak Summary with Statistics Name: Naftopidil

	Units
1	mcg
2	mcg
3	mcg
4	mcg
5	mcg
Mean	
Std. Dev.	
% RSD	

Component Summary For Retention Time
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil Robust_ 0.8 ml _inj 1	1	W2489 ChA	10	6.106
2	Naftopidil Robust_ 0.8 ml _inj 2	2	W2489 ChA	10	6.115
3	Naftopidil Robust_ 0.8 ml _inj 3	3	W2489 ChA	10	6.076
4	Naftopidil Robust_ 0.8 ml _inj 4	4	W2489 ChA	10	6.056
5	Naftopidil Robust_ 0.8 ml _inj 5	5	W2489 ChA	10	6.062
Mean					6.083
Std. Dev.					0.026
% RSD					0.4

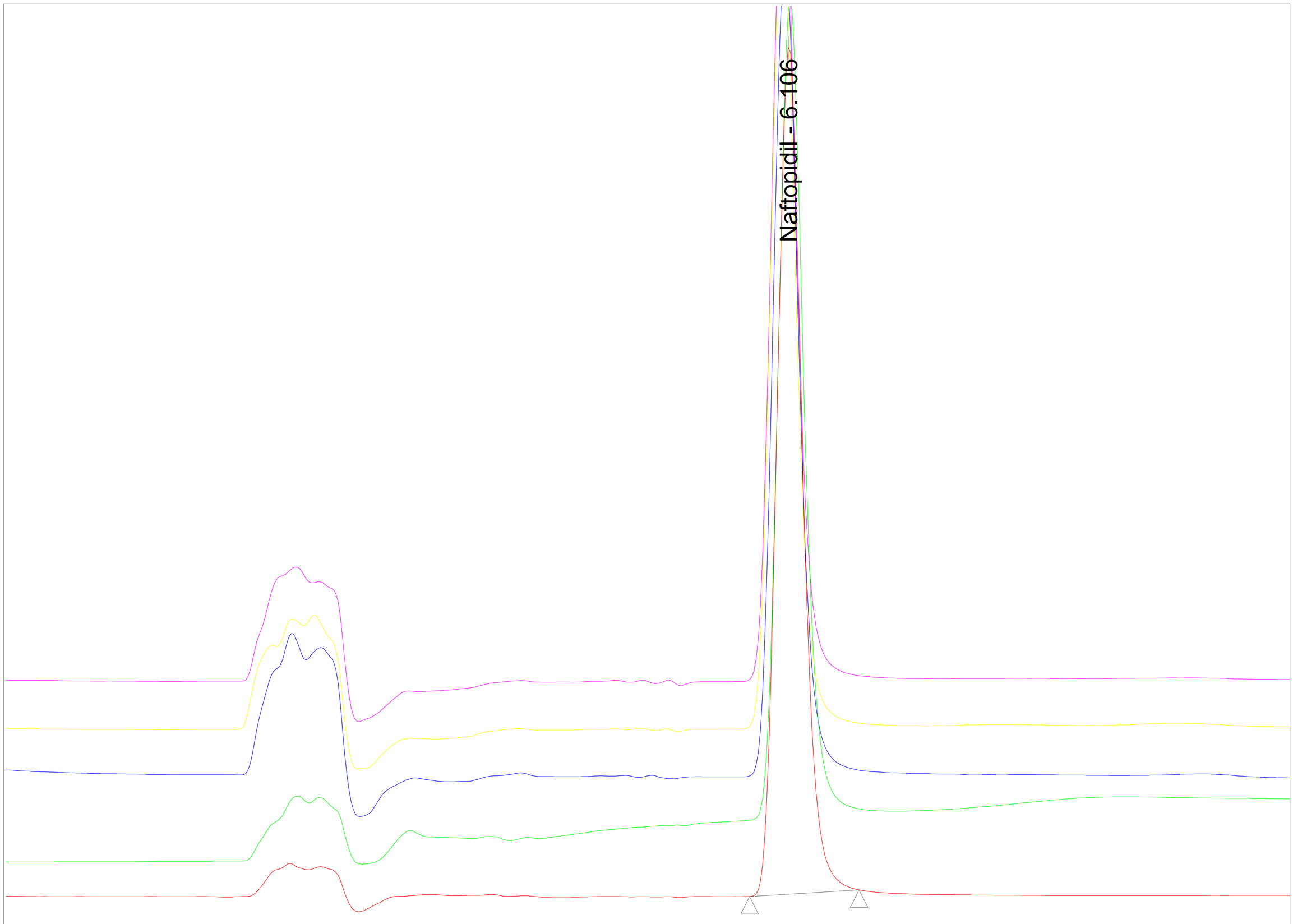
Component Summary For Area
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil Robust_ 0.8 ml _inj 1	1	W2489 ChA	10	907816
2	Naftopidil Robust_ 0.8 ml _inj 2	2	W2489 ChA	10	917729
3	Naftopidil Robust_ 0.8 ml _inj 3	3	W2489 ChA	10	901840
4	Naftopidil Robust_ 0.8 ml _inj 4	4	W2489 ChA	10	924316
5	Naftopidil Robust_ 0.8 ml _inj 5	5	W2489 ChA	10	921297
Mean					914600
Std. Dev.					9459
% RSD					1.0

Component Summary For Amount
Channel: W2489 ChA

	Sample Name	Inj	Channel	Vial	Naftopidil
1	Naftopidil Robust_ 0.8 ml _inj 1	1	W2489 ChA	10	50.000
2	Naftopidil Robust_ 0.8 ml _inj 2	2	W2489 ChA	10	50.000
3	Naftopidil Robust_ 0.8 ml _inj 3	3	W2489 ChA	10	50.000
4	Naftopidil Robust_ 0.8 ml _inj 4	4	W2489 ChA	10	50.000
5	Naftopidil Robust_ 0.8 ml _inj 5	5	W2489 ChA	10	50.000
Mean					50.000
Std. Dev.					0.000
% RSD					0.0

Peak Summary Report



Sample Name: Naftopidil Robust_ 0.8 ml _inj 1; Date Acquired: 4/4/2016 4:07:36 PM IST; Vial: 10; Injection: 1
Sample Name: Naftopidil Robust_ 0.8 ml _inj 2; Date Acquired: 4/4/2016 4:19:04 PM IST; Vial: 10; Injection: 2
Sample Name: Naftopidil Robust_ 0.8 ml _inj 3; Date Acquired: 4/4/2016 4:30:08 PM IST; Vial: 10; Injection: 3
Sample Name: Naftopidil Robust_ 0.8 ml _inj 4; Date Acquired: 4/4/2016 4:41:14 PM IST; Vial: 10; Injection: 4
Sample Name: Naftopidil Robust_ 0.8 ml _inj 5; Date Acquired: 4/4/2016 4:52:14 PM IST; Vial: 10; Injection: 5

Peak Summary with Statistics
Name: Naftopidil

	Sample Name	Vial	Inj	Name	Retention Time (min)	Area	% Area	Height	Amount
1	Naftopidil Robust_ 0.8 ml _inj 1	10	1	Naftopidil	6.106	907816	100.00	66092	50.0
2	Naftopidil Robust_ 0.8 ml _inj 2	10	2	Naftopidil	6.115	917729	100.00	66179	50.0
3	Naftopidil Robust_ 0.8 ml _inj 5	10	5	Naftopidil	6.062	921297	100.00	67109	50.0
4	Naftopidil Robust_ 0.8 ml _inj 4	10	4	Naftopidil	6.056	924316	100.00	67248	50.0
5	Naftopidil Robust_ 0.8 ml _inj 3	10	3	Naftopidil	6.076	901840	100.00	65416	50.0

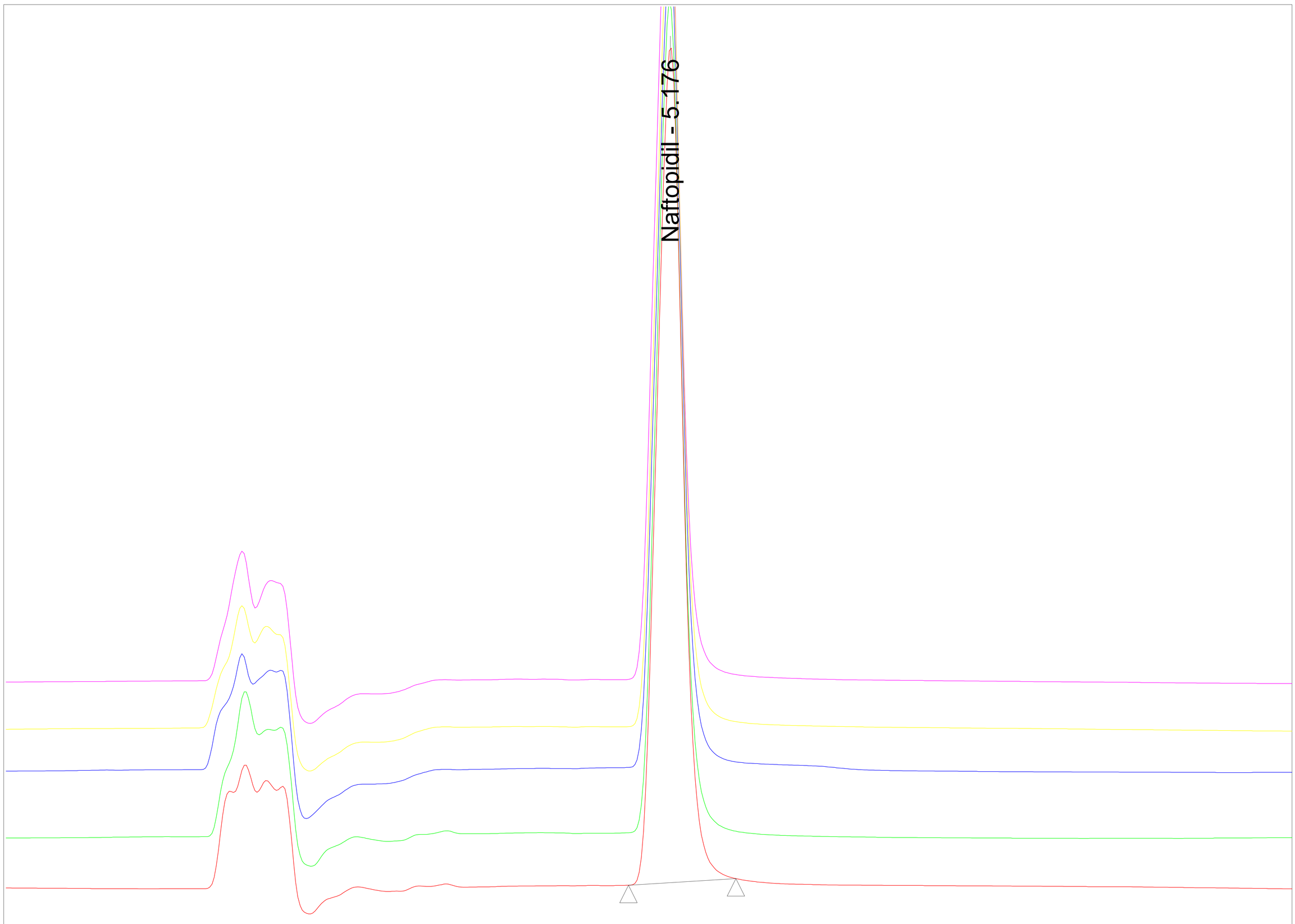
Peak Summary with Statistics
Name: Naftopidil

	Sample Name	Vial	Inj	Name	Retention Time (min)	Area	% Area	Height	Amount
Mean					6.083				
Std. Dev.					0.026				
% RSD					0.44				

Peak Summary with Statistics Name: Naftopidil

	Units
1	mcg
2	mcg
3	mcg
4	mcg
5	mcg
Mean	
Std. Dev.	
% RSD	

Peak Summary Report



Sample Name: Naftopidil Robust_ 1.2 ml _inj 1; Date Acquired: 4/4/2016 5:05:34 PM IST; Vial: 11; Injection: 1
Sample Name: Naftopidil Robust_ 1.2 ml _inj 2; Date Acquired: 4/4/2016 5:17:41 PM IST; Vial: 11; Injection: 2
Sample Name: Naftopidil Robust_ 1.2 ml _inj 3; Date Acquired: 4/4/2016 5:28:45 PM IST; Vial: 11; Injection: 3
Sample Name: Naftopidil Robust_ 1.2 ml _inj 4; Date Acquired: 4/4/2016 5:39:42 PM IST; Vial: 11; Injection: 4
Sample Name: Naftopidil Robust_ 1.2 ml _inj 5; Date Acquired: 4/4/2016 5:50:59 PM IST; Vial: 11; Injection: 5

Peak Summary with Statistics
Name: Naftopidil

	Sample Name	Vial	Inj	Name	Retention Time (min)	Area	% Area	Height	Amount
1	Naftopidil Robust_ 1.2 ml _inj 1	11	1	Naftopidil	5.176	808416	100.00	57917	50.0
2	Naftopidil Robust_ 1.2 ml _inj 2	11	2	Naftopidil	5.170	805167	100.00	58896	50.0
3	Naftopidil Robust_ 1.2 ml _inj 5	11	5	Naftopidil	5.160	806354	100.00	58301	50.0
4	Naftopidil Robust_ 1.2 ml _inj 4	11	4	Naftopidil	5.168	785601	100.00	56955	50.0
5	Naftopidil Robust_ 1.2 ml _inj 3	11	3	Naftopidil	5.170	802597	100.00	57959	50.0

Peak Summary with Statistics
Name: Naftopidil

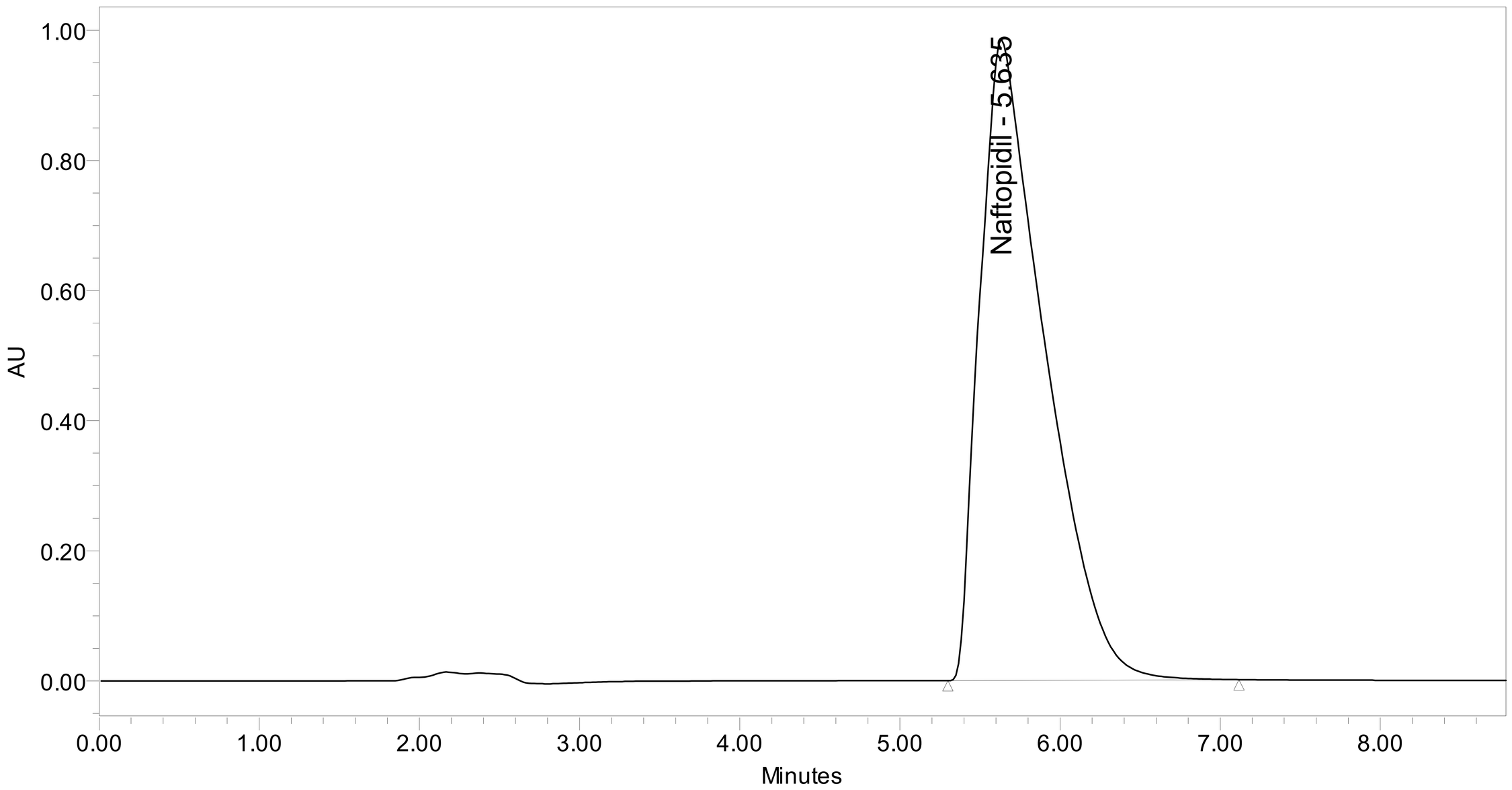
	Sample Name	Vial	Inj	Name	Retention Time (min)	Area	% Area	Height	Amount
Mean					5.169				
Std. Dev.					0.006				
% RSD					0.12				

Peak Summary with Statistics Name: Naftopidil

	Units
1	mcg
2	mcg
3	mcg
4	mcg
5	mcg
Mean	
Std. Dev.	
% RSD	

SAMPLE INFORMATION

Sample Name:	Naftopidil deg. Acidic.API	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	
Vial:	4	Acq. Method Set:	Naftopidil
Injection #:	1	Processing Method:	Naftopidil
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 282nm
Date Acquired:	5/19/2016 1:19:25 PM IST		
Date Processed:	5/19/2016 3:30:35 PM IST		



	Peak Name	RT	Area	% Area	Height	Amount	Units
1	Naftopidil	5.635	27808904	100.00	985564	50.000	mcg

System Suitability Separation Results

	Name	RT	Resolution	Symmetry Factor	USP Plate Count	USP Tailing
1	Naftopidil	5.635			695.783275	1.828319

SAMPLE INFORMATION

Sample Name:	Naftopidil deg. Alkaline API	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	
Vial:	6	Acq. Method Set:	Naftopidil
Injection #:	1	Processing Method:	Naftopidil
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 282nm
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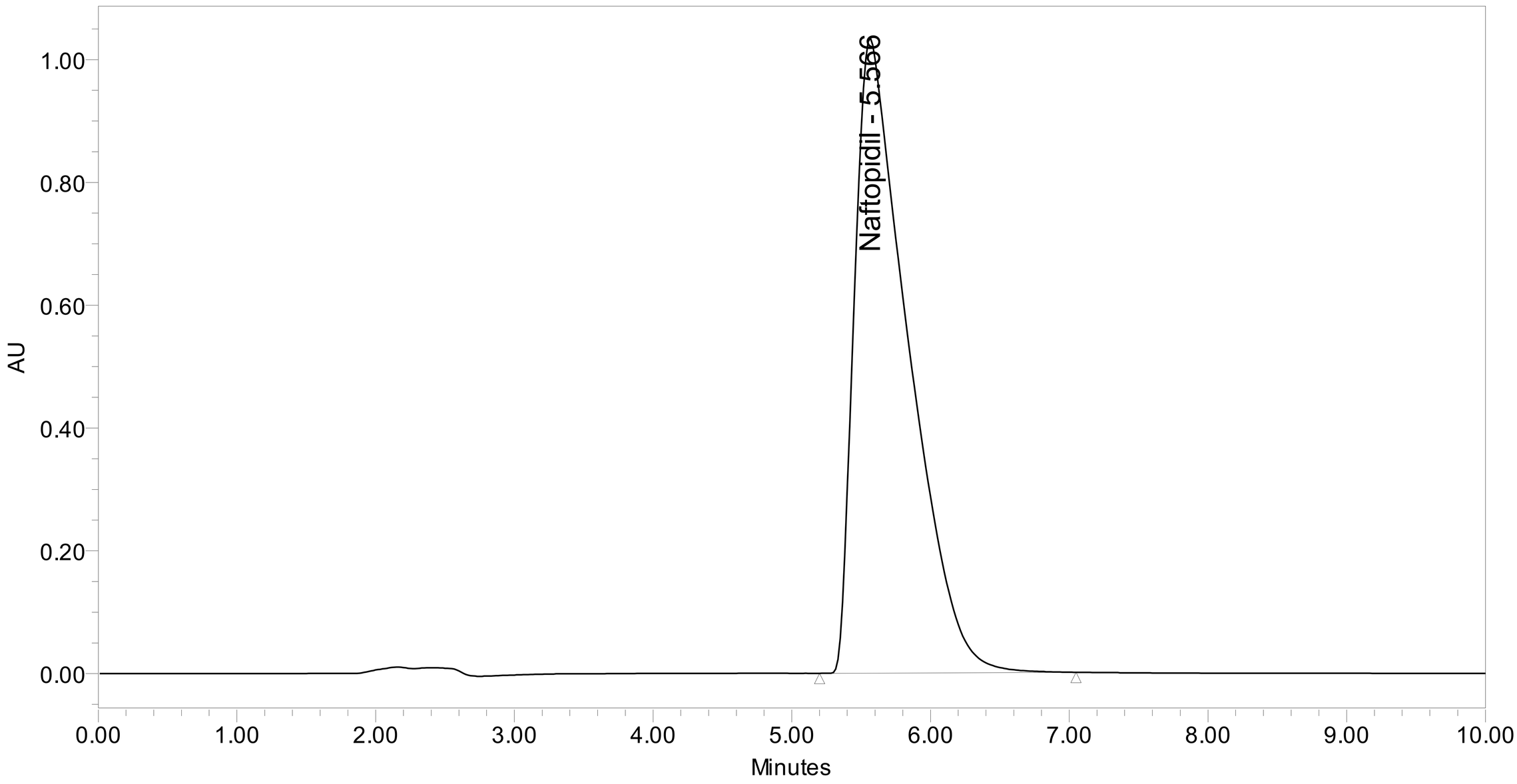

	Peak Name	RT	Area	% Area	Height	Amount	Units
1	Naftopidil	5.566	27805775	100.00	1034255	50.000	mcg

System Suitability Separation Results

	Name	RT	Resolution	Symmetry Factor	USP Plate Count	USP Tailing
1	Naftopidil	5.566			809.801087	2.065524

SAMPLE INFORMATION

Sample Name:	Naftopidil deg. Alkaline Tablet	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	
Vial:	7	Acq. Method Set:	Naftopidil
Injection #:	1	Processing Method:	Naftopidil
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 282nm
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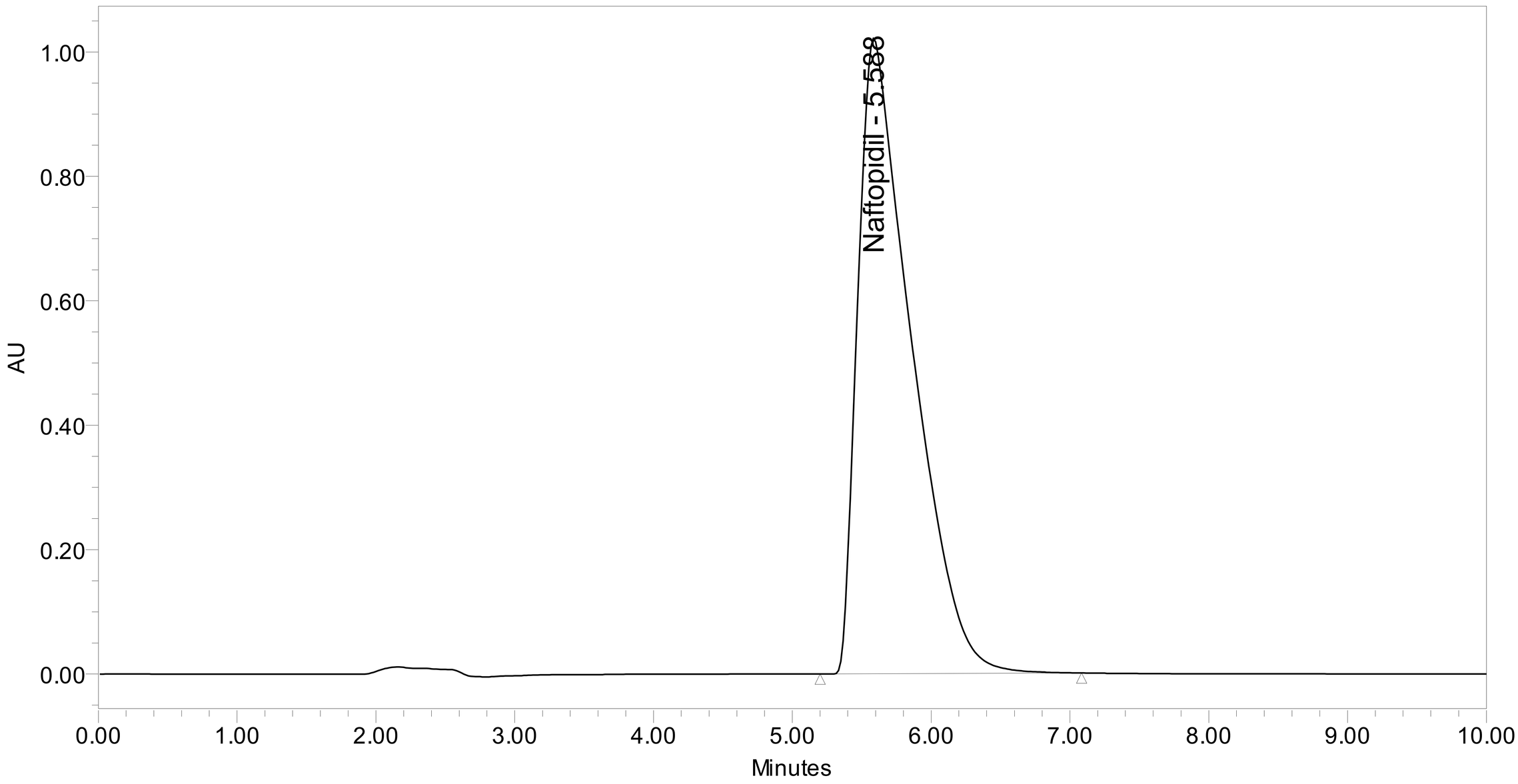


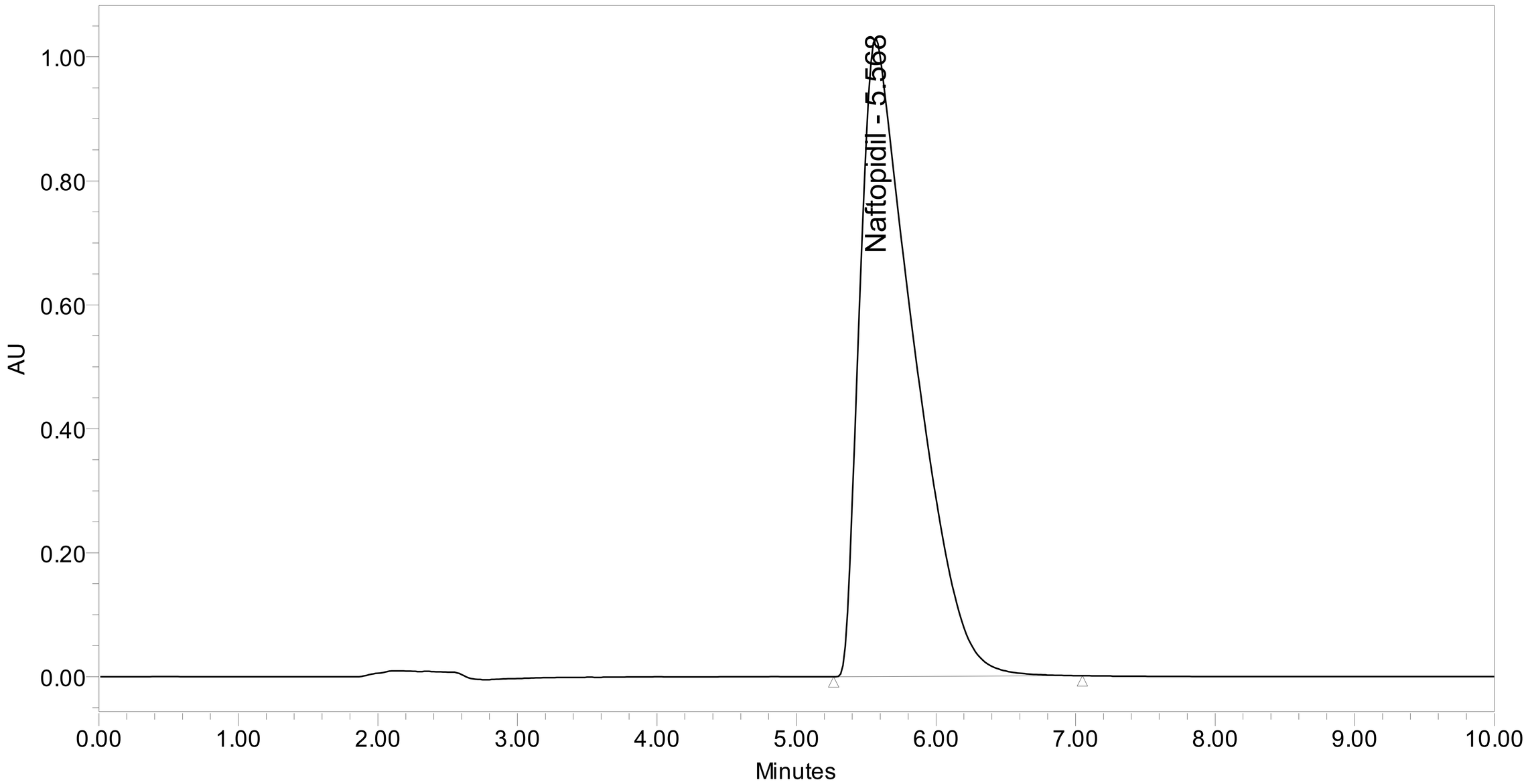
	Peak Name	RT	Area	% Area	Height	Amount	Units
1	Naftopidil	5.588	27441009	100.00	1022316	194.272	mcg

System Suitability Separation Results

	Name	RT	Resolution	Symmetry Factor	USP Plate Count	USP Tailing
1	Naftopidil	5.588			809.135888	2.025702

SAMPLE INFORMATION

Sample Name:	Naftopidil deg. Oxidation Tablet	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	
Vial:	9	Acq. Method Set:	Naftopidil
Injection #:	1	Processing Method:	Naftopidil
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 282nm
Date Acquired:	5/19/2016 2:14:45 PM IST		
Date Processed:	5/19/2016 3:30:35 PM IST		



	Peak Name	RT	Area	% Area	Height	Amount	Units
1	Naftopidil	5.568	27607822	100.00	1030694	186.072	mcg

System Suitability Separation Results

	Name	RT	Resolution	Symmetry Factor	USP Plate Count	USP Tailing
1	Naftopidil	5.568			810.609071	2.066112

SAMPLE INFORMATION

Sample Name:	Naftopidil deg. UV API	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	
Vial:	10	Acq. Method Set:	Naftopidil
Injection #:	1	Processing Method:	Naftopidil
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 282nm
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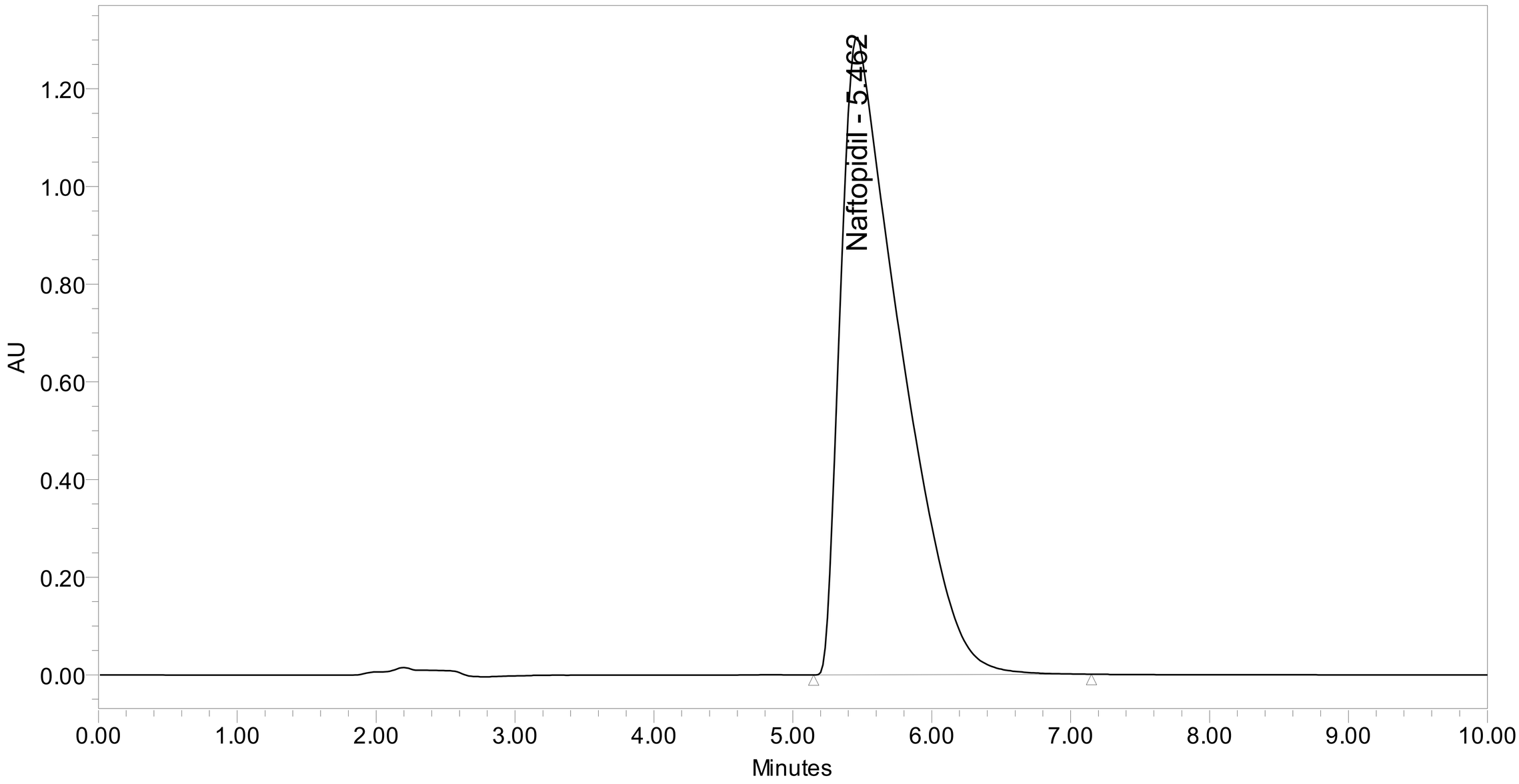
Date Processed:	5/19/2016 3:30:35 PM IST		



	Peak Name	RT	Area	% Area	Height	Amount	Units
1	Naftopidil	5.462	38975476	100.00	1305746	50.000	mcg

System Suitability Separation Results

	Name	RT	Resolution	Symmetry Factor	USP Plate Count	USP Tailing
1	Naftopidil	5.462			625.816080	2.226135

SAMPLE INFORMATION

Sample Name:	Naftopidil deg. UV Tablet	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	
Vial:	11	Acq. Method Set:	Naftopidil
Injection #:	1	Processing Method:	Naftopidil
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 282nm
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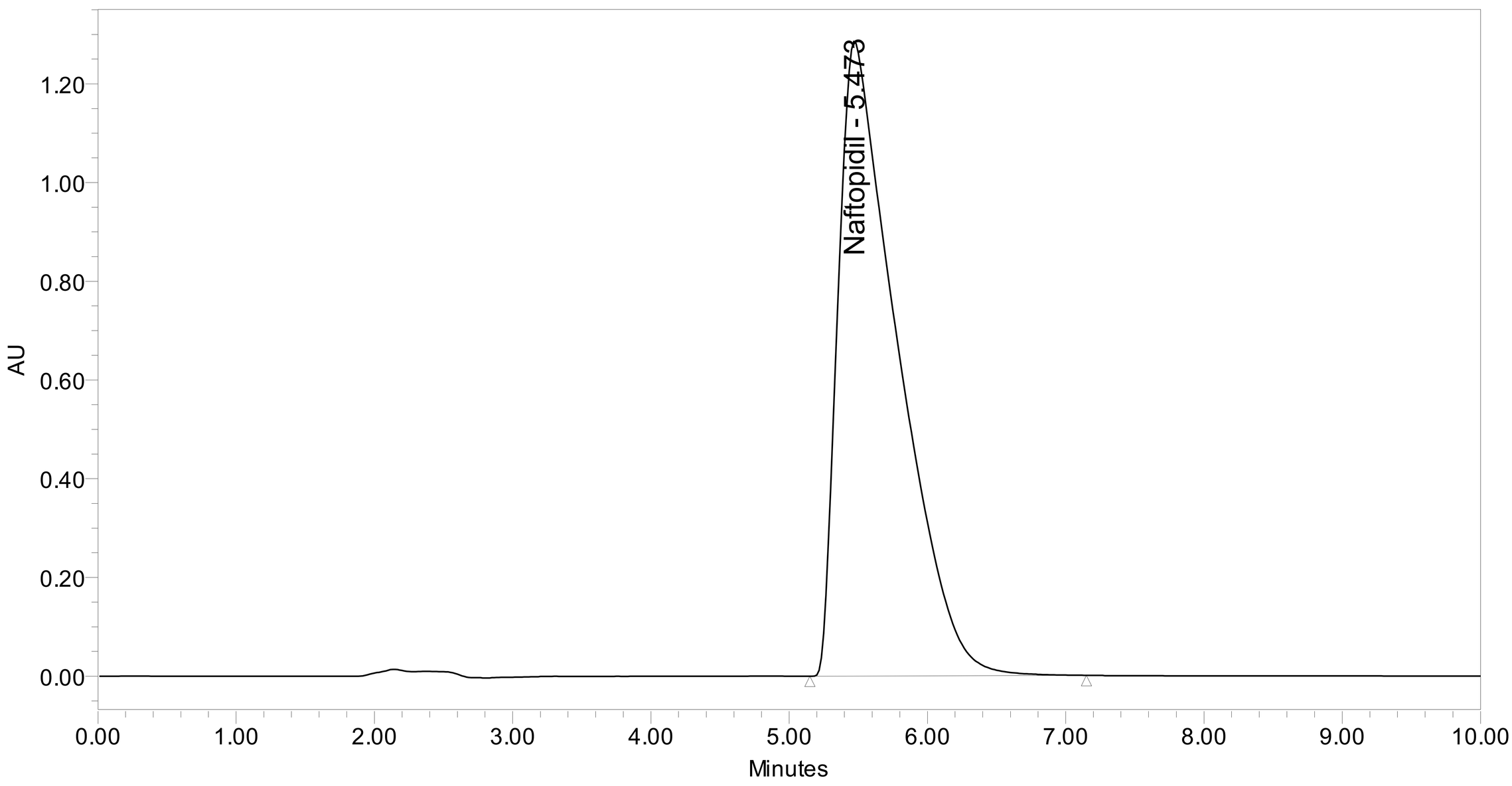

	Peak Name	RT	Area	% Area	Height	Amount	Units
1	Naftopidil	5.473	38369520	100.00	1287444	252.207	mcg

System Suitability Separation Results

	Name	RT	Resolution	Symmetry Factor	USP Plate Count	USP Tailing
1	Naftopidil	5.473			625.586443	2.174900

SAMPLE INFORMATION

Sample Name:	Naftopidil deg. Dry Heat Tablet	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	
Vial:	13	Acq. Method Set:	Naftopidil
Injection #:	1	Processing Method:	Naftopidil
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 282nm
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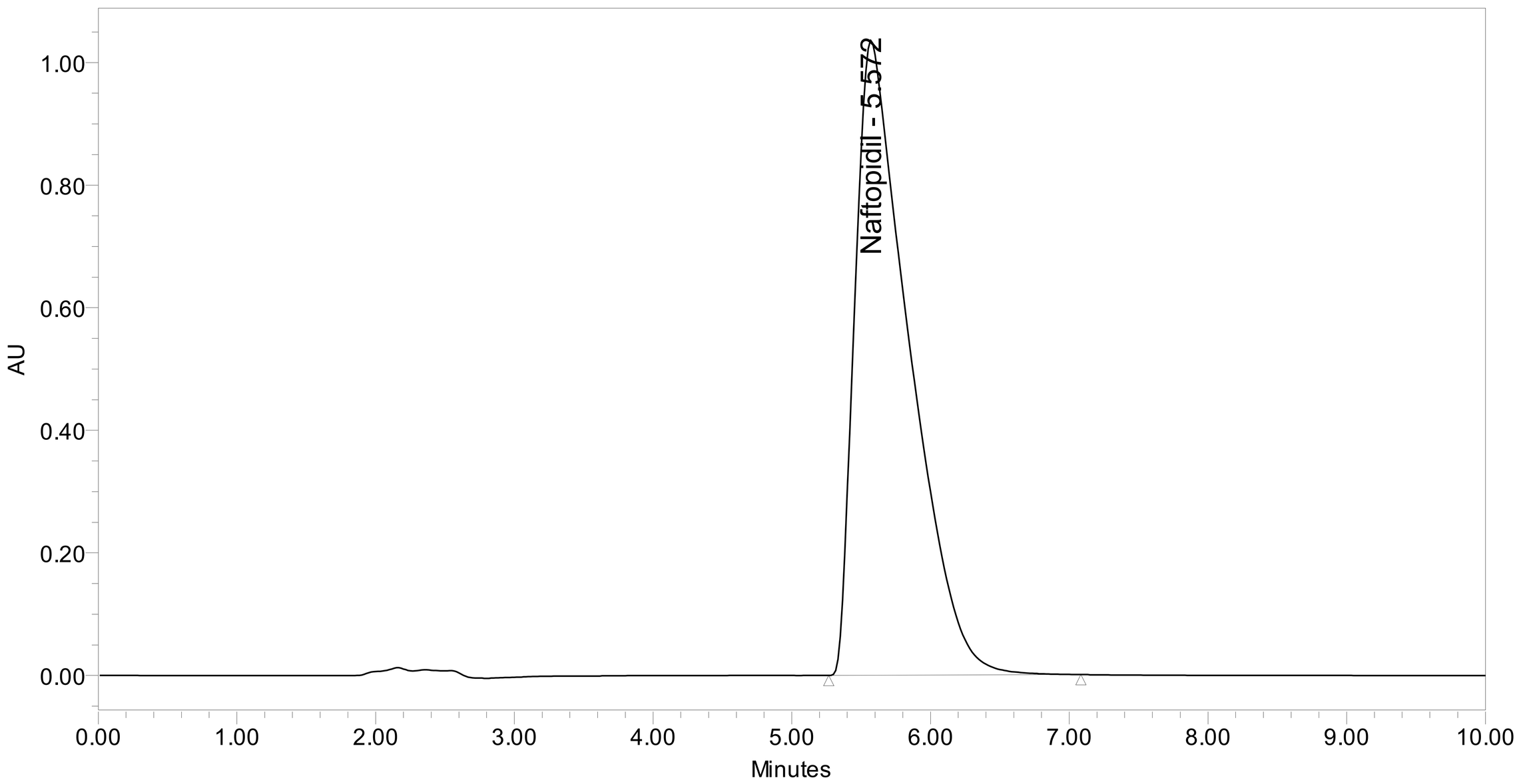

	Peak Name	RT	Area	% Area	Height	Amount	Units
1	Naftopidil	5.572	28197090	100.00	1037236	189.694	mcg

System Suitability Separation Results

	Name	RT	Resolution	Symmetry Factor	USP Plate Count	USP Tailing
1	Naftopidil	5.572			786.642629	2.012986

SAMPLE INFORMATION

Sample Name:	Naftopidil deg. Humid cond. API	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	
Vial:	14	Acq. Method Set:	Naftopidil
Injection #:	1	Processing Method:	Naftopidil
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 282nm
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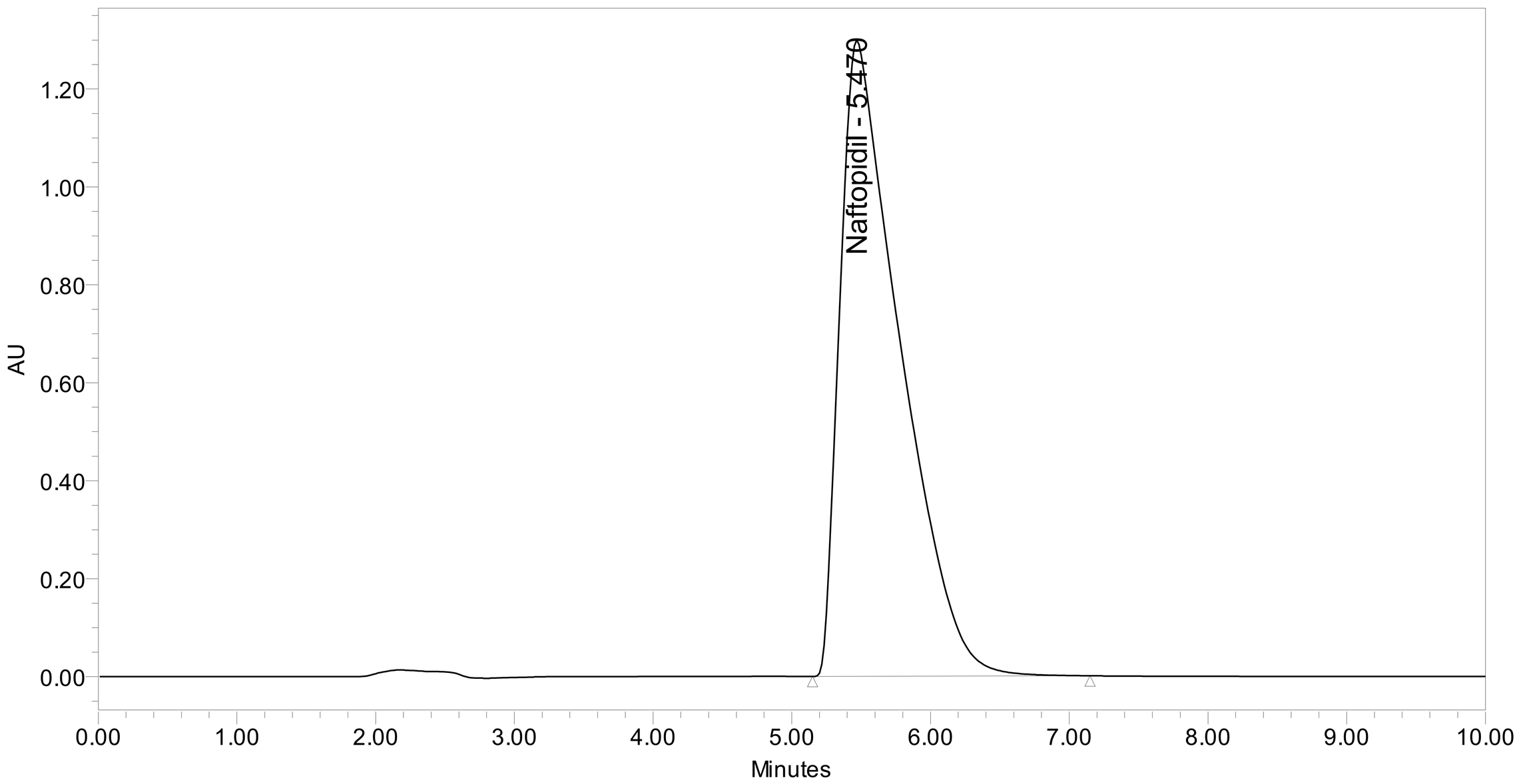

	Peak Name	RT	Area	% Area	Height	Amount	Units
1	Naftopidil	5.470	39074992	100.00	1299407	50.000	mcg

System Suitability Separation Results

	Name	RT	Resolution	Symmetry Factor	USP Plate Count	USP Tailing
1	Naftopidil	5.470			613.580133	2.142752

SAMPLE INFORMATION

Sample Name:	Naftopidil deg. Humid cond. Tabl	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	
Vial:	15	Acq. Method Set:	Naftopidil
Injection #:	1	Processing Method:	Naftopidil
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 282nm
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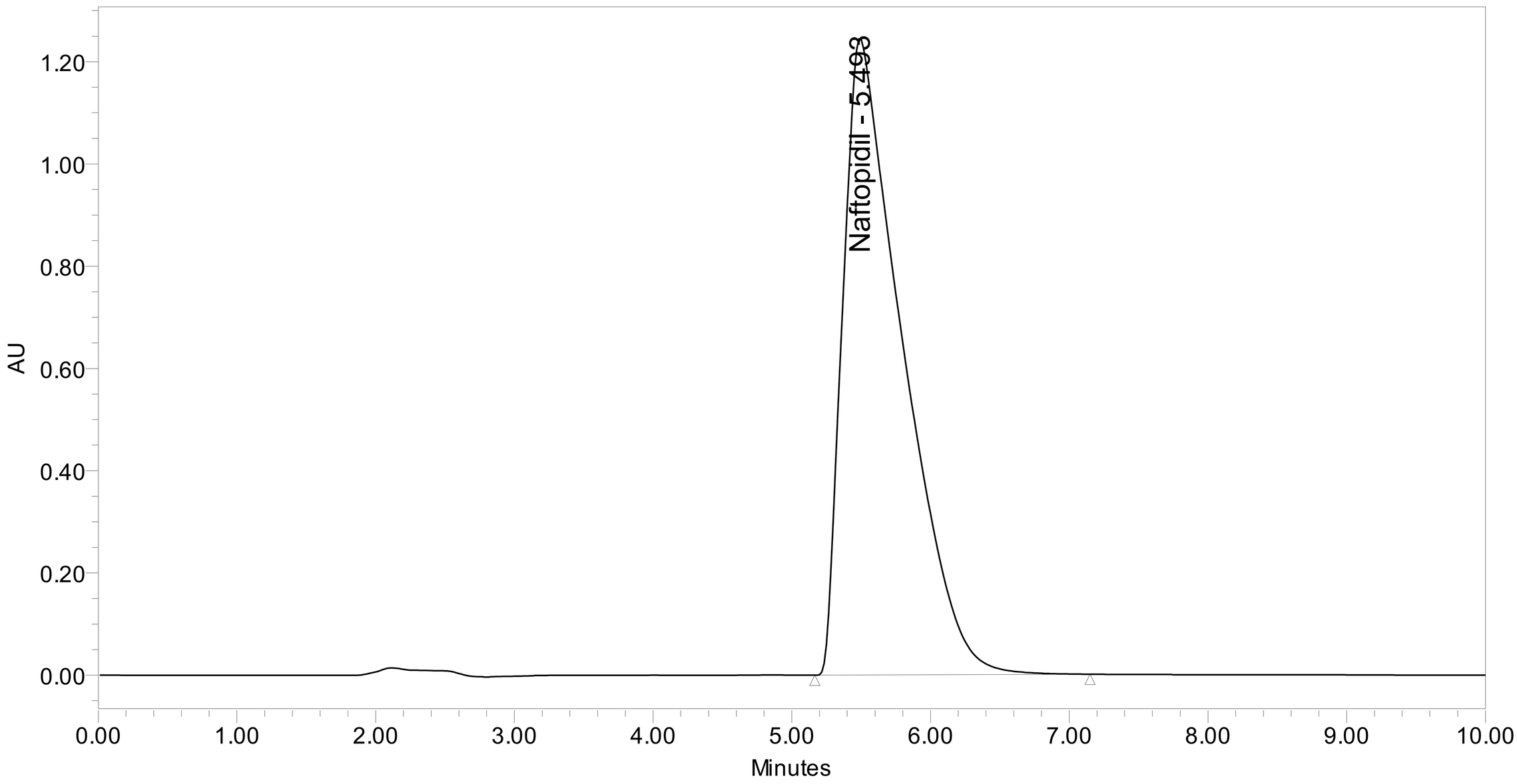

	Peak Name	RT	Area	% Area	Height	Amount	Units
1	Naftopidil	5.493	37075568	100.00	1247799	244.256	mcg

System Suitability Separation Results

	Name	RT	Resolution	Symmetry Factor	USP Plate Count	USP Tailing
1	Naftopidil	5.493			622.251714	2.073567

Chapter 9

REFERENCE

9.0 REFERENCES:

1. ICH Q2A Validation of Analytical procedure : Methadology International Conference on Harmonization , Geneva October, 1994.

2. Masumori N "Naftopidil for the treatment of urinary symptoms in patients with benign prostatic hyperplasia",Theraputic and Clinical Risk Management,2011;7:227-228. doi: 10.2147/TCRM.S13883

3. http://www.simcere.com/simcere/productpic/enpro_25_1_200759164904.pdf

4. http://www.drugsupdate.com/generic/view/1254/Naftopidil

5. Chatwal G.R and Anand K.S;instrumental methods of chemical analysis,5th Edn Himalaya publishing House,mumbai, 2002: 2-149

6. Sethi, P.D.,Quantitative Analysis of Drugs in Pharamceutical Formulation,3rded.,CBS Publishers and Distributors, 1997; 1-29 :50-64

7. Behn F, Mir TS, Scholz H, Laer S, "HPLC quantification of Carvedilol in small volumes of plasma in small children", *Chromatographia*, 2001; 53: 641-644.
8. Xiawen L, Yunying Z, Mu Y, Yinxiang S, "Determination of naftopidil enantiomers in rat plasma using chiral solid phases and pre-column derivatization high-performance liquid chromatography", *Journal of Chromatography* B, 2012; 907: 140-145.
9. Jain PS, Bobade KD, Bari PR, Girase DS, Surana SJ, "Development and validation of analytical method for Naftopidil in human plasma by LC–MS/MS", *Arabian Journal of Chemistry,* 2013 http://dx.doi.org/10.1016/j.arabjc.2013.06.020.
10. Farthering MJ, Alstead EM, Abrams SM, Haug G, Johnston A, Hermann R, Niebch G, Russ P, Molz KH,Turner P, "Pharmacokinectics of naftopidil, a novel antihypertensive drug, in patients with hepatic dysfunction", *Postgradute Medical Journal,* 1994; 70: 363- 366.
11. Satheeshkumar N, Spandana1 V, Shantikumar S, Srinivas R, " Experimental design approach to optimize stability indicating liquid chromatography method for the determination of Naftopidil in its bulk and tablet dosage form", *Journal of Young Pharmacists*, 2014; 6: 1-7.
12. Pavan AB, Vijayalakshmi M, Manoj PM, "Devolopment and validation of RP-HPLC method for the estimation of Naftopidil in bulk and dosage form", *International Journal of Research in Pharmacy and Chemistry,* 2012; 2: 2231-2781.

13. Raghu Babu K, Sarma S, Raju GMJ, Sarma GVS, Aruna Kumari N, "Simple and Stability Indicating RP-HPLC Assay Method Development And Validation Naftodipil by RP-HPLC in Bulk and Dosage Form", *World Journal of Pharmacy and Pharmaceutical Science,* 2015; 4: 900-909.

14. Georg N, Harald O, Eberhard B, "High-performance liquid chromatography of naftopidil, a novel antihypertensive drug, and two metabolites in human plasma", *Journal of Chromatography B,* 1990; 534: 247-252.

15. Blessy M, RuchiD.Patel, PrajeshN.Prajapati, Y.K.Agrawal, "Development of forced degradation and stability indicating studies of drugs—A review" Journal of Pharmaceutical Analysis 2014; 4(3): 159–165