ESTIMATION OF ALFUZOSIN HYDROCHLORIDE BY UV-VISIBLE SPECTROPHOTOMETRY AND RP-HPLC

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Submitted by

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Under the Guidance of

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CERTIFICATE

This is to certify that the research work entitled "ESTIMATION OF ALFUZOSIN HYDROCHLORIDE BY UV-VISIBLE SPECTROPHOTOMETRY AND RP-HPLC" submitted to The Tamil Nadu Dr.M.G.R. Medical University in partial fulfillment for the award of the Degree of Master of Pharmacy (Pharmaceutical Analysis) was carried out by P.SURESH in the Department of Pharmaceutical Analysis under my direct guidance and supervision during the academic year 2007- 2008.

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CERTIFICATE

This is to certify that the dissertation entitled "ESTIMATION OF ALFUZOSIN HYDROCHLORIDE BY UV-VISIBLE SPECTROPHOTOMETRY AND RP-HPLC" is the bonafide research work carried out by P. SURESH in the Department of Pharmaceutical Analysis, Adhiparasakthi College of Pharmacy, Melmaruvathur which is affiliated to The Tamil Nadu Dr.M.G.R. Medical University under the guidance of Mr. M. SUGUMARAN M.Pharm., Assistant Professor, Department of Pharmaceutical Analysis, Adhiparasakthi College of Pharmacy, during the academic year 2007- 2008.

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LIST OF ABBREVIATIONS USED

α	-	Alpha
% RSD	-	Percentage Relative Standard Deviation
%	-	Percentage
μ	-	Micron
λ	-	Lambda
λ_{max}	-	Absorption maximum
AUC	-	Area Under Curve
BPH	-	Benign Prostatic Hyperplasia
mM	-	milimole
C_{max}	-	Concentration maximum
$CYP_3 A_4$	-	Cytochrome $CYP_3 A_4$ enzyme
min	-	minutes
HC1	-	Hydrochloric acid
HPLC	-	High Performance Liquid Chromatography
HPTLC	-	High Performance Thin Layer Chromatography
IP	-	Indian Pharmacopoeia
IR	-	Infrared
L	-	Litre
LOD	-	Limit of Detection
LOQ	-	Limit of Quantification
μg	-	microgram
mg	-	milligram
ml	-	milliliter

NaOH	-	Sodium Hydroxide.
ng	-	nanogram
r	-	Regression coefficient
RP-HPLC	-	Reverse Phase High Performance Liquid Chromatography
RT	-	Retention Time
SD	-	Standard Deviation
SE	-	Standard Error
USP	-	United States Pharmacopeia
UV	-	Ultraviolet
NMR	-	Nulclear Magnetic Resonance spectroscopy
ORD	-	Optical Rotary Dispersion
CD	-	Circular Dichrosium
DTA	-	Differential Thermal Analysis
DSC	-	Differential Scanning Calorimetry
BP	-	British Pharmacopoeia
THF	-	Tetra Hydro Furan
FDA	-	Food and Drug Association
Kg	-	Kilogram

Dedicated to my Ever Loving Parents

Introduction

1. INTRODUCTION

Analytical chemistry has played a major role in the changes facing the pharmaceutical industry today. Traditionally viewed as a service organization, the analytical department has become a significant parameter in the drug development process. Indeed, the demand for analytical data has become a critical path activity for selection of molecule for full development. The pharmaceutical analysis plays a major role in assuring, identity, safety, efficacy, purity and quality of a drug product. The need for pharmaceutical analysis is driven largely by regulatory requirements. (Satinder Ahuja, 2005; Takeru Higuchi, 2002)

The discipline of analytical chemistry consists of,

1.Qualitative analysis: - which reveals the identity of the element and compound in a sample. (Alexeyev, 1994)

2.Quantitative analysis: - indicate the amount of individual element or compound present in sample.

1.1. Methods of quantitative analysis (Devaraj Rao, 2004)

Under the quantitative analysis, the procedures of quantitative analytical chemistry are applied to the analysis of material used in pharmaceuticals.

There are various methods for quantitative analysis of pharmaceuticals. In general the components are related characteristically to some physical property and on the basis of that, the quantity is estimated. They are classified into the following types:

1.1.1 Chemical Methods

1.1.1.1 Volumetric or Titrimetric

a. Acid-base or Neutralizations Titrations

b. Oxidation-Redution or Redox titrations

c. Precipitation titration

d. Complexometric titrations

e. Non-aqueous titrations

1.1.1.2. Gravimetric

1.1.1.3. Geometric

1.1.2. Instrumental or physico-chemical methods

Instrumental methods are based on the relation between the content and corresponding physical or physico-chemical properties of the system being analyzed

PHYSICAL PROPERTY MEASURED	INSTRUMENTAL METHODS BASED ON MEASUREMENT OF PROPERTY
Absorption radiation	Spectrophotometry (x-ray, UV, Visible, IR)
Emission of radiation	NMR.
Rotation of radiation	Fluorimetry, Flame photometry
Electrical Potential	Polarimetry, ORD, CD.
Electrical conductance	Potentiometry, Chrono-potentiometry
Electrical current	Conductometry.
Thermal properties	Polarography, Amperometry.
	DTA, DSC etc.

1.1.2.1. Chromatographic methods

i) Thin layer chromatography (TLC)

ii) Paper chromatography

iii) Column chromatography

iv) Gas chromatography

v) High Performance Liquid Chromatography (HPLC)

1.1.2.2. Hyphenated techniques

i) GC-MS (Gas chromatography – Mass spectrometry)

ii) LC-MS (Liquid chromatography – Mass spectrometry)

iii)GC-IR (Gas chromatography – Infrared spectroscopy)

iv) ICP-MS (Inductively coupled plasma – Mass spectrometry)

1.1.3. Microbiological methods

1.1.4. Biological methods

- **Factors affecting the choice of analytical methods** (Mendham, 2002)
- The type of analysis required.
- Problem arising from the nature of the material
- Possible interference from components of the material other than those of interest.
- The concentration range which needs to be investigated.
- The accuracy required.
- The facilities available.
- The time required for complete analysis.
- Similar type of analysis performed.

1.2. UV- SPECTROSCOPY (Atherden, 1996; Satinder Ahuja, 2005)

UV-absorptions are mainly electronic in nature and are associated with resonating structure in the molecule. It involves the measurement of the amount of UV (200-400nm) radiation absorbed by a substance in solution. UV - absorption is an essential tool for qualitative and quantitative determination of a single component drug or isolated extract. The UV quantitative determination generally performed in solution is based on the Beer-Lambert's law. .

UV region of the electromagnetic spectrum is frequently subdivided into far vacuum UV region (10 – 200 nm) and near UV region (200 – 400 nm). The utilization of near UV absorption spectra as an analytical tool has increased in recent years, because of commercial availability of the UV spectrophotometers which are recording the spectrum in this region.

Quantitative determination of single drug substance in a nonchromophoric solvent such as aqueous or alcohol solution may be a good application for the UV method. In a method development study, solubility, stability studies are performed with the UV technique. UV is extensively used for HPLC detection.

1.3. VISIBLE SPECTROPHOTOMETRY

(Mendham, 2002**;** Satinder Ahuja, 2005)

Visible spectrometry is identical to UV spectrometry, with the exception of the wavelength, which are ranges from 400-800 nm. Most drug substances are colorless; however, a color product may be formed with specific agent, as a result of chemical reaction. Quantitative determination of the colored compound is based on

this principle for drug assay. This technique was used quite extensively during the early stages of pharmaceutical analysis in the years of 1950 to 1960.

Advantages of the colorimetric method include the following:

1. Inexpensive equipment(colorimeter) used for analysis(useful for field test)

2. Selective detection of a colored compound formed. (Selective chemical reagent reacting with the compound of interest alone, e.g. Functional group analysis).

3. Enhanced detection sensitivity for compounds with low UV-characteristics.

   Another method of forming a color compound (subsequently separated by extraction) is the dye-salt method. In an ion pair reaction, a colored complex of the drug with a dye of opposite polarity is formed which is extracted into the organic layer and determined colorimetrically.

Colorimetric analysis should satisfy following criteria.

1. The color reaction should be specific.

2. Proportionality change between color and concentration.

3. Color should be stable to permit an accurate reading

4. Reproducible results should be notified.

5. Solution must be free from precipitate.

6. The color reaction should be highly sensitive.

1.3.1. The Absorption laws (Sharma, 1980)

There are two laws which govern the absorption of light by the molecules. These are,

i . Beer's law

ii. Lambert's law

i. Beer's law

This law states that, when a beam of monochromatic radiation is passed through a solution of an absorbing substance, the rate of decrease in intensity of transmitted radiation with thickness of the absorbing solution is directly proportional to the intensity of incident radiation as well as the concentration of the solution.

ii. Lambert's law

It states that, when a beam of monochromatic radiation passes through a homogenous absorbing medium, the rate of decrease in intensity of transmitted radiation with thickness of absorbing medium is directly proportional to the intensity of the incident radiation.

1.3.2. Choice of solvent (Robert, 2006; Sharma, 2000)

The choice of solvent that is used in an assay in the ultraviolet-visible region is based upon two criteria. First, of course, the sample must be soluble in the solvent; the solvent must be transparent to the radiation of the wavelength in which the study is conducted.

The solvents can be used at wavelengths that are longer than the cutoff wavelength. A most suitable solvent is the one which doesn't itself absorb in the region under investigation. A dilute solution of the sample is always prepared for the spectral analysis. The absorbance of solvents should be checked before use, because the presence of small amounts of impurity may give rise to appreciable absorption in the sample. For ultra violet and visible spectroscopy, ethanol, water and cyclohexane serve as the best solvents.

1.3.3. Detectors (Willard, 1986)

A detector is a transducer, converting electro magnetic radiation into an electron flow and subsequently into a current flow or voltage in the read out circuit. Many times the photo current requires amplification, particularly when measuring low levels of radiant energy. There are single element detectors such as solid state photo diodes, photo emissive tubes and photo multiplier tubes and multiple element detectors such as solid state array detectors. Important characteristics of any type of detector are spectral sensitivity, wave length response, gain and response time.

1.3.4. Quantitative analysis (Beckett and stenlake, 2002)

The assay of an absorbing substance may be quickly carried out by preparing a solution in a transparent solvent and measuring its absorbance at a suitable wavelength. The wavelength normally selected is a wavelength of maximum absorption (λ_{max}), where small errors in setting the wavelength scale have little effects on the measured absorbance.

a. Assay of substances in single component samples

Absorption spectroscopy is one of the most useful tools available to the chemist for quantitative analysis. The most important characteristics of photometric and spectrophotometric methods are high selectivity and ease of convenience. Quantitative analysis (assay of an absorbing substance) can be done using following methods.

- Use of A (1%, 1cm) values
- Use of calibration graph (multiple standard methods)
- By single or double point standardization method.

i. Use of A (1%, 1cm) values

This method can be used for estimation of drug from formulations or raw material, when reference standard not available. The use of standard A (1%, 1cm) value avoids the need to prepare a standard solution of the reference substance in order to determine its absorption.

ii. Use of calibration graph

In this method, a calibration curve is plotted using concentration (X-axis) Vs absorbance (Y-axis) with the value of 5 or more standard solutions. A straight line is drawn through maximum number of points. This method is called as line of best fit. By interpolating the absorbance of the sample solution and using the calibration curve, the concentration of the drug amount and percentage purity can be calculated.

iii. Single or double point standardization

The procedure involves the measurement of the absorbance of a sample solution and standard solution. The standard and the sample solution are prepared in similar manner; ideally the concentration of the standard solution should be close to that of the sample solution. The concentration of the substance in the sample is calculated using following formula.

$$C_{test} = A_{test} / A_{std} \times C_{std}$$

Where,

C_{test} and C_{std} are the concentration of the sample and standard solutions respectively.

A_{test} and A_{std} are the absorbance of the sample and standard solutions respectively.

In double point standardization, the concentration of one of the standard solution is greater than that of the sample, while the other standard solution has a lower concentration than the sample solution. The concentration of the substance in the sample solution is given by

$$C_{test} = \frac{(A_{test} - A_{std1})(C_{std1}-C_{std2}) + C_{std1}(A_{std1}-A_{std2})}{A_{std1}-A_{std2}}$$

Where,

C_{std} is the concentration of the standard solution.

A_{test} and A_{std} are the absorbance of the sample and standard solution respectively.

std_1 and std_2 are the more concentrated standard and less concentrated standard respectively.

b. Assay of substances in multi component samples

The spectrophotometric assay of drugs rarely involves the measurement of absorbance of samples containing only one absorbing component. The pharmaceutical analyst frequently encounters the situation, where the concentration of one or more substances is required in samples known to contain other absorbing substances which potentially interfere in the assay. Unwanted absorption from these sources is termed irrelevant absorption and if not removed, imparts systematic errors to the assay of the drug in the sample. A number of modifications to the simple spectrophotometric procedure for single-component samples is available to the analyst, which may eliminate certain sources of interferences and permit the accurate determination of one or all of the absorbing components.

The basis of all the spectrophotometric technique for multicomponent samples is the property that at all wavelengths,

a) The absorbance of a solution is the sum of absorbances of the individual components; or

b) The measured absorbance is the difference between the total absorbance of the solution in the sample cell & that of the solution in the reference (blank) cell.

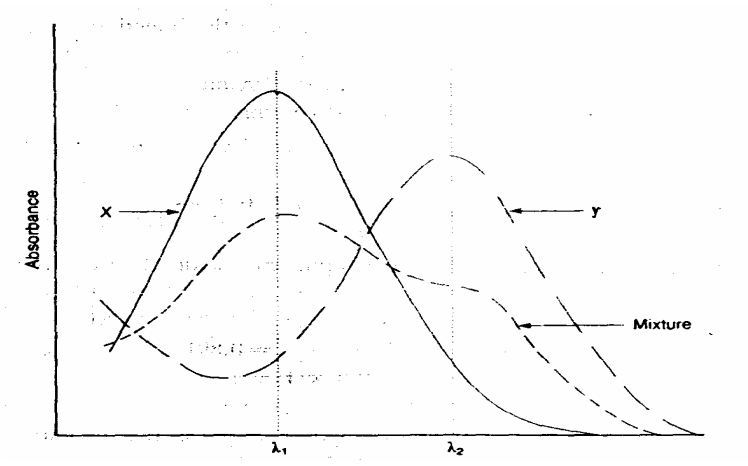
The determination of the multi-component samples can be done by using the following methods:

- Assay of a single-component sample
- Assay using absorbance corrected for interference

- Assay after solvent extraction of the sample
    - ❖ Simultaneous equations method
    - ❖ Absorbance ratio method (Q-Analysis)
    - ❖ Geometric correction method
    - ❖ Orthogonal polynomial method
    - ❖ Difference spectrophotometry
    - ❖ Derivative spectrophometry
    - ❖ Chemical derivatisation

i. Simultaneous equation method

If a sample contains two, absorbing drugs (X and Y) each of which absorbs at the λ_{max} of the other as shown in Fig, it may be possible to determine both drugs by the technique of simultaneous equations (Vierodt's method).



The information required is:

a) the absorptivities of X at λ_1 and λ_2, ax_1 and ax_2, respectively

(b) the absorptivities of Y at λ_1 and λ_2, ay_1 and ay_2, respectively

(c) the absorbance of the diluted sample at λ_1 and λ_2, A_1 and A_2 respectively.

Let Cx and Cy, be the concentrations of X and Y respectively in the diluted sample.

Two equations are constructed based upon the fact that at λ_1 and λ_2 the absorbance of the mixture is the sum of the individual absorbance's of x and y,

At λ_1 $\quad$ $\mathbf{A_1 = ax_1bc_x + ay_1bc_y}$ -------- (1)

At λ_2 $\quad$ $\mathbf{A_2 = ax_2bc_x + ay_2bc_y}$ --------- (2)

For measurement in 1cm cells, b = 1.

Rearrange eq.(2)

$$Cy = \frac{A2 - ax2cx}{ay2}$$

Substituting for Cy in eq,(1) and rearranging gives

$$Cx = \frac{\mathbf{A_2ay_1 - A_1ay_2}}{\mathbf{ax_2ay_1 — ax_1ay_2}} \quad \text{---------(3)}$$

and

$$Cy = \frac{\mathbf{A_2ay_1 - A_1ay_2}}{\mathbf{ax_2ay_1 — ax_1ay_2}} \quad \text{---------(4)}$$

As an exercise you should derive modified equations containing a symbol (b) for path length, for application in situations where A_1, and A_2 are measured in cells other than 1 cm path length.

$$\frac{\mathbf{A_2/A_1}}{\mathbf{ax_2/ax_1}} \quad \text{and} \quad \frac{\mathbf{ay_2/ay_1}}{\mathbf{A_2/A_1}}$$

Criteria for obtaining maximum precision, based upon absorbance ratios, have been suggested that place limits on the relative concentrations of the components of the mixture. The criteria are that the ratios should lie outside the range 0.1-2.0 for the precise determination of Y and X respectively. These criteria are satisfied only when the λ_{max} of the two components are reasonably dissimilar. An additional criterion is that the two components do not interact chemically, thereby negating the initial assumption that the total absorbance is the sum of the individual absorbance. The additivity of the absorbance should always be confirmed in the development of a new application of this technique. The British Pharmacopoeia assay of quinine-related alkaloids and cinchonine-related alkaloids in cinchona bark is based, upon this technique.

ii. Absorbance ratio method

The absorbance ratio method is a modification of the simultaneous equations procedure. It depends on the property that, for a substance which obeys Beer's law at all wavelengths, the ratio of absorbances at any two wavelengths is a constant value independent of concentration or pathlength. For example, two different dilutions of the same substance give the same absorbance ratio A_1/A_2, 2.0. In the USP, this ratio is referred to as a Q value. The British Pharmacopoeia also uses a ratio of absorbances at specified wavelengths in certain confirmatory tests of identity. For example,

Cyanocobalamin exhibits three λ_{max} at 278 nm, 361 nm and 550 nm. The A_{360}/A_{550} is required to be 3.30 ± 0.15 and the A_{361}/A_{278} to be 1.79 ± 0.09.

iii. Geometric correction method

A number of mathematical correction procedures have been developed which reduce or eliminate the background irrelevant absorption that may be present in samples of biological origin. The simplest of these procedures is the three-point geometric procedure, which may be applied if the irrelevant absorption is linear at the three wavelengths selected.

iv. Orthogonal polynomial method

The technique of orthogonal polynomials is another mathematical correction procedure which involves more complex calculations than the three –point correction procedure. The basis of the method is that an absorption spectrum may be represented in terms of orthogonal functions as follows:

$$A(\lambda) = p_0P_0(\lambda) + p_1P_1(\lambda) + p_2P_2(\lambda) \ldots p_nP_n(\lambda)$$

Where A denotes the absorbance at wavelength λ belonging to a set of n + 1 equally spaced wavelengths at which the orthogonal polynomials, $P_0(\lambda)$, $P_1(\lambda)$, $P_2(\lambda)$ . . . $P_n(\lambda)$ are each defined.

v. Area under the curve method

From the spectra obtained for calculating the simultaneous equation, the area under the curve were selected at a particular wavelength range for both the drugs were each drug have its absorption. The "X" values of the drugs were determined at the selected AUC range. The "X" value is the ratio of area under the curve at the selected wavelength range with the concentration of the component in mg/ml. These "X" values were the mean of six independent determinations. A set of two simultaneous

equations were obtained by using mean "X" values. And further calculations are carried out to obtain the concentration of each drug present in the sample.

vi. Derivative spectrophotometry

Derivative spectrophotometry involves the conversion of a normal spectrum to its first, second or higher derivative spectrum. The transformations that occur in the derivative spectra are understood by reference to a Gaussian band which represents an ideal absorption band. In the context of derivative spectrophotometry, the normal absorption spectrum is referred to as the fundamental, zeroth order or D° spectrum.

The first derivative (D^1) spectrum is a plot of the ratio of change of absorbance with wavelength against wavelength, i.e. a plot of the slope of the fundamental spectrum against wavelength or a plot of $dA/d\lambda$ Vs λ. At $\lambda 2$ and $\lambda 4$, the maximum positive and maximum negative slope respectively in the D°. Spectrums correspond with maximum and a minimum respectively in the D^1 spectrum. The λ_{max} at λ_3 is a wavelength of zero slope and gives $dA/d\lambda$ 0, i.e. a cross-over point, in the D1. Spectrum.

The zeroth (a), first (b) and second (c) derivative spectra of a Gaussian band.The second derivative (D^2) spectrum is a plot of the curvature of the D° spectrum against wavelength or a plot of $d^2A/d\lambda^2$ Vs λ. The maximum negative curvature at λ_3 in the D° spectrum gives a minimum in the D^2 spectrum, and at λ_1 and λ_5 the maximum positive curvature in the D° spectrum gives two small maxima called 'satellite' bands in the D^2 spectrum. At λ_2 and λ_4 the wavelengths of maximum slope and zero curvature in the D° spectrum correspond with cross-over points in the D^2 spectrum.

In summary, the first derivative spectrum of an absorption band is characterised by a maximum, a minimum, and a cross-over point at the λ_{max} of the absorption band. The-second derivative spectrum is characterized by two satellite maxima and an inverted band of which the minimum corresponds to the λ_{max} of the fundamental band. As an exercise, you should construct third and fourth derivative spectra (i.e. plots of $d^3A/d\lambda^3$ and $d^4A/d\lambda^4$ respectively against wavelength) of the fundamental spectrum.

These spectral transformations confer two principal advantages on derivative spectrophotometry. Firstly, an even order spectrum is of narrower spectral bandwidth than its fundamental spectrum. A derivative spectrum therefore shows better resolution of overlapping bands than the fundamental spectrum and may permit the accurate determination of the λ_{max} of the individual bands. Secondly, derivative spectrophotometry discriminates in favors of substances of narrow spectral bandwidth against broad bandwidth substances. This is because 'the derivative amplitude (D), i.e. the distance from a maximum to a minimum, is inversely proportional to the fundamental spectral bandwidth (14') raised to the power (n) of the derivative order.

Thus, $$\mathbf{D \alpha (1/W)^n}$$

Consequently, substances of narrow spectral bandwidth display larger derivative amplitudes than those of broad bandwidth substances.

(a) The individual spectra of two components X and Y in admixture and their combined spectrum (b) The second derivative spectrum of the mixture showing improved resolution of the individual bands.

These advantages of derivative spectrophotometry, enhanced resolution and bandwidth discrimination, permit the selective determination of certain absorbing substances in samples in which non-specific interference may prohibit the application

of simple spectrophotometeric methods. For example, benzenoid drugs such as Ephedrine Hydrochloride, displaying fine structure of narrow spectral bandwidth in the region 240—270 nm, are both weakly absorbing (A about 15) and formulated at a relatively low dose in solid dosage preparations (typically 1—50 mg/ unit dose). The high excipient/drug ratio and high sample weight required for the assay may introduce into simple spectrophotometric procedures serious irrelevant absorption from the formulation excipients. Second derivative spectrophotometry discriminates in favour of the narrow bands of the fine structure of the benzenoid drugs and eliminates the broad band absorption of the excipients. All the amplitudes in the derivative spectrum are proportional' to the concentration of the analyte, provided that Beer's Law is obeyed by the fundamental spectrum. The measured value in a quantitative assay is the largest amplitude that is unaffected by the presence of other, absorbing components of the sample.

The enhanced resolution and bandwidth discrimination, increases with increasing derivative order. However, it is also found that the concomitant increase in electronic noise. Inherent in the generation of the higher order spectra, and the consequent reduction of the signal-to-noise ratio, place serious practical limitations on the higher order spectra.

1.4. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

(Satinder Ahuja, 2005; Lloyd, 1997)

HPLC has contributed many successes in quality control and in product development in the pharmaceutical industry. The UV detector coupling with HPLC equipment is the most important analytical instrument for quality control, quality assurance and in-process control in pharmaceutical analysis. HPLC is a basic and

reliable analytical tool for analytical study because of the high resolution capacity, accuracy and reproducibility of the equipment.

High performance liquid chromatography is a convenient separation technique used for wide types of samples, with exceptional resolving power, speed and nano molecular detection levels. It is presently used in pharmaceutical research and developments in the following ways:

- To purify synthetic or natural products
- To characterize the metabolites.
- To assay active ingredients, impurities, degradation products and in dissolution assays.
- In pharmacodynamic and pharmacokinetic studies.

Chromatography encompasses a diverse group of methods that are utilized for the separation of closely related components of mixtures. In all chromatographic separations, the sample is transported within the mobile phases, which may be a gas (GC), a liquid (LC), or a supercritical fluid (SFC). In column chromatography, the stationary phase is contained within a narrow tube through which the mobile phase is forced by gravity or under pressure. The components of the mixture to be analyzed distribute themselves between the mobile phase and stationary phase in varying proportions. Compounds that interact strongly with the stationary phase migrate very slowly with the mobile phase; in contrast, compounds that are weakly retained by the packing material migrate rapidly with the mobile phase. As a consequence of the differences in mobility between the individual components of a mixture, the sample components are separated into discrete bands (or zones) that emerge from the column at specific 'retention times'. These bands may be identified qualitatively and /or further analyzed quantitatively using an appropriate detector.

Early, liquid chromatography was carried out in glass columns with diameter of 1 to 0.5 cm and length of 50 to500 cm. The average diameter of the solid stationary phase particles was usually in the 100 to 200 micron range. Recent technology has allowed for the development of packing material with relatively small particles size diameter (3-10 μ). This technology resulted in the development of columns with very high efficiencies, and consequently has involved the use of more sophisticated instrumentation to perform at increased pressures and flows; hence the term High Performance Liquid Chromatography (HPLC) was given to this technique.

The typical HPLC separation is based on the selective distribution of analytes between a liquid mobile phase and an immiscible stationary phase. The sample is first introduced by means of an injection port into the mobile phase stream that is delivered by a high-pressure pump.

The method is popular because it is non-destructive and may be applied to thermally liable compounds (unlike GC); it is also a very sensitive technique since it incorporates a wide choice of detection methods. With the use of post-column derivatisation methods, selectivity and detection limits are improved. HPLC can easily be extended to trace determination of compounds that do not usually provide adequate detector response. The wide applicability of HPLC as a separation method makes it valuable separation tool in many scientific fields.

1.4.1. INSTRUMENTATION

The essential parts of the high performance liquid chromatography are the solvent reservoir, tubing, pump, injection device, column, detector, and recorder.

1.4.1.1. Solvent reservoir (James, W. Munson, 2001)

The composition of the reserviour should render it inert to a variety of aqueous and non aqueous mobile phases. Stainless steel and glass are obviously the best choices. The volume should be greater than 500ml.

1.4.1.2. Sample injection system

The injection of the sample onto the column creates some unique problem because of the high pressure involved in HPLC. While septum injectors can be used in HPLC: the loop injector fined the widest use. Direct sample introduction via syringe in HPLC is the simplest form of injection.

1.4.1.3. Pumps (Willard, 1986)

1. Syringe pump (screw driven) (or) Displacement pump
2. Reciprocating pump
    - Single piston reciprocating pump
    - Dual head piston reciprocating pump
    - Reciprocating diaphragm pump
3. Pneumatic pump (or) Constant pressure pump
    - Direct pressure pump
    - Amplifier pump

1.4.1.4 Mobile Phases (Sethi, 2001)

In most of the cases, the mobile phases are binary or ternary system. It is advisable to filter each solvent before mixing. HPLC grade solvents rarely have particulate matter and HPLC system has in-line filters as further guard, but as part of preventive maintenance, the chromatographer should resort to filtration of the solvents. Even minute particulate matter not visible to the naked eye can pass various problems due to accumulation of the same at the head of column such as change in K'

value, non-producible flow rate, decrease in selectivity, spurious peaks, increase in back pressure, irreversible adsorption and shortening of column life.

1.4.1.5 Columns (Sethi, 2001; Skoog, 2004)

Column is often referred to as the heart of HPLC separation process. Liquid chromatographic columns are ordinary constructed from smooth-bore stainless stell tubing. Stable high performance column is essential requisite for rugged and reproducible method. Columns commercially available differ among the suppliers or even the same source in terms of plate number (N), band symmetry, band spacing, retention and life time. For high separation efficiency, large number of theoretical plates is necessary per unit length of the column. Analytical column and guard columns are most common columns for HPLC.

1.4.1.6. Detectors (Sethi, 2001; Willard, 1986)

Detector is the eye of LC system and measures the compounds after the separation on the column. Before the first sample is injected, during method development the chromatographer must ensure that the detector so selected is capable of responding to changes in the concentration of all the components in the sample with adequate sensitivity even to measure trace substances.

There are basically two types of detectors.

- Bulk property detectors
- Solute property detectors

The Bulk property detectors function based on some bulk property of the eluent, such as refractive index and are not suitable for gradient elution and are usually less sensitive than solute property detectors. The solute property detectors perform by measuring some type of physical or chemical property.

1.4.2. METHODS IN CHROMATOGRAPHY (Sethi, 2001)

- Adsorption chromatography
  - Normal phase chromatography
  - Reserved phase chromatography
  - Ion exchange chromatography
  - Affinity phase chromatography
  - Hydrophobic Interaction Chromatography (HIC)
- Partition chromatography
  - Gas chromatography
  - Liquid partition chromatography
- Size Exclusion Chromatography (SEC)
  - Gel permeation chromatography
  - Gel filtration chromatography

Normal phase chromatography

Mechanism**:** Retention by interaction of the stationary phase's polar surface with polar parts of the sample molecules.

Stationary phase**:** In the normal phase chromatography, the nature of stationary phase is polar. e.g: SiO_2, Al_2O_3, $-NH_2$, -CN, -Diol and $-NO_2$.

Mobile phase**:** Nature of mobile phase is non-polar. e.g: Hectane, hexane, cyclo hexane, $CHCl_3$, CH_2Cl_2, dioxane and methanol.

Application**:** Separation of non-ionic, non-polar to medium polar substances.

Reverse phase chromatography

Mechanism**:** Retention by interaction of the stationary phase's non-polar hydrocarbon chain with non-polar parts of the sample molecules.

Stationary Phase**:** Nature of stationary phase is non-polar. e.g: n-octadecyl (C_{18}), n-octyl (C_8), n-hexyl (C_6) and ethyl (C_2).

Mobile phase**:** Nature of mobile phase is polar. e.g: Methanol or acetronitrile / water or buffer (some times with additives of THF or dioxane)

(Rule of thumb**:** Increase of water content by 10% results in doubling the K' value.)

Application**:** Separation of non-ionic and ion forming non-polar to medium polar substances (carboxylic acids -> hydrocarbons). If ion forming substances (as carboxylic acid) are to be separated, p^H control by using buffers is necessary.

1.4.3. THEORY OF OPERATION

1.4.3.1. Column efficiency (Llyod, 1997; Willard, 1986)

Column efficiency refers to the performance of the stationary phase to accomplish particular separations. Column efficiency is a funtion of different separation variables. This entails how well the column is packed and its kinetic performance. The efficiency of a column can be measured by several methods which may or may not be affected by chromatographic anomalies, such as "tailing" or appearance of a "front". This is important because many chromatographic peaks do not appear in the preferred shape of normal Gaussian distribution. For this reason, efficiency can be an enigmatic value since manufacturers may use different methods in determining the efficiency of their columns.

Calculation of column efficiency value

All the following methods use this formula that measures N, or number of theoretical plates:

$$N = \frac{a\, t_r^2}{W}$$

a = constant dependent on height, where peak width is measured

t_r = retention time

W = peak width

1.4.3.2. Inflection method

Calculation is based upon inflection point which appears at 60.7% of the peak height for a normal Gaussian peak. At this point, the width of the peak is equivalent to two standard deviation units. Any asymmetrical aspect of a peak should not affect this calculation since the width is measured above the anomalous occurrence (i.e., tailing or fronting).

1.4.3.3. Half-peak height method

As the name suggests, the measurement is based upon the width at 50% of peak height. For the same reason as inflection method, this measurement is not affected by asymmetry; however, this method is more reproducible from person to person since width at 50% peak height is less prone to be varied.

1.4.3.4. Tangent method

Tangent lines are drawn on each side of the peak and the width is the distance between the two lines at the base of the peak. Therefore, it is more sensitive to asymmetrical peaks and variation in efficiency values is usually seen from user to user.

1.4.3.5. Sigma Method

This method measures peak width at decreasing levels of peak height. Thus the three sigma method measures width at 32.4% of peak height, the four sigma method measures at 13.4%, and the five sigma method measures at 4.4%.The five sigma method is most sensitive to asymmetry because the width is measured at the lowest point.

1.4.3.6. Height/Area method

This method utilizes the fact that the area of a peak is a function of its height and standard deviation. To determine the efficiency, values for peak height and area are used in a different formula:

$$N = \frac{2\pi (ht_r^2)}{A^2}$$

Where,

N = No. of theoretical plates

h = peak height

t_r = retention time

A = peak area

1.4.3.7. Moment method

This method entails disregarding peak shape and expresses parameters of the peak in tactical moments. The zero moment, μ_0 is the mean and occurs at the center of the peak area. The first moment, μ_1 is the mean and occurs at the center of the peak (which is the maximum peak height in normal Gaussian peaks). The second moment

μ_2 is the variance of the peak. This is a detailed method, where appropriate data systems are needed. For a more detailed discussion, a reference is provided.

1.4.4. QUANTITATIVE ANALYSIS

Quantification involves the measurement of peak height or peak area. To determine the concentration of a compound, the peak area or height is plotted vs the concentration of the substance. For well resolved peaks, both peak height and area are proportional to the concentration. Four different calibration methods used in quantitative analysis are,

a. Normalized peak area

b. External standard addition method

c. Internal standard addition method

d. Standard addition method

a. Normalized peak area

After completion of a run and the integration of all significant peaks in the chromatogram, the total peak area can be calculated. This technique is widely used to estimate the relative amount of small impurities or degradation compound in a purified material. The area present of any individual peak is referred to as the normalized peak area. The technique of normalized peak area is actually not a calibration method, since there is no comparison to a known amount for any peak in the chromatogram.

b. External standard calibration

The most general method for determining the concentration of an unknown sample is to construct a calibration plot using external standards. Standards are prepared at known concentrations. A fixed volume of each standard solution is

injected and analyzed, and the peak responses are plotted Vs concentration. The standard solutions are referred to as external standards, since they are prepared and analyzed in separate chromatograms from those of the unknown samples. Unknown samples are then prepared, injected and analyzed in exactly the same manner.

c. Internal standard calibration

Another technique for calibration involves the addition of an internal standard to the calibration solutions and samples. The internal standard is a different compound form the analyte, but one that is well resolved in the separation. The internal standard can compensate for changes in sample size or concentration to instrumental variations. With the internal standard method, a calibration plot is produced by preparing and analyzing calibration solutions containing different concentration of the compound of interest with a fixed concentration of the internal added.

The Internal standard must complies the following requirements :

- Well resolved from the compound of interest and other peaks.
- Similar retention (k) to the analyte.
- Should not be in the original sample
- Should mimic the analyte in any sample preparation steps.
- Does not have to be chemically similar to analyte.
- Commercially available in high purity.
- Stable and unreactive with sample or mobile phase.
- Should have similar detector response to the analyte for the concentration used
- It must be separated from all compounds of interest in the separation.

d. Method of standard addition

A calibration standard ideally should be prepared in a blank matrix of drug formulation components without the drug substance or an animal without added compound usually can be used for standard calibration solutions. The method of standard addition is most often used in trace analysis. In this approach, different weights of analyte(s) are added to the sample matrix, which initially contains an unknown concentration of the analyte. Extrapolation of a plot of response found for the standard addition calibration concentration to zero concentration defines the original concentration in the unspiked sample.

1.5. ICH GUIDELINES FOR ANALYTICAL METHOD VALIDATION

(Code Q2A, 1996; Code Q2B, 1996)

Method validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Methods need to be validated or revalidated,

- Before their introduction into routine use
- Whenever the conditions change for which the method has been validated, e.g., instruments with different characteristics.
- Whenever the method is changed, and the change is outside the original scope of the method. The International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceutical for human use has developed a consensus text on the validation of analytical procedures. The document includes definitions for eight validation characteristics.

The parameters as defined by the ICH and by other organizations

- Specificity
- Selectivity
- Precision
  - Repeatability
  - Intermediate precision
  - Reproducibility
- Accuracy
- Linearity
- Range
- Limit of detection
- Limit of quantization
- Robustness
- Ruggedness

1.5.1. SPECIFICITY

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedures.

An investigation of specificity should be conducted during the validation of identification tests, the determination of impurities and the assay. The procedures used to demonstrate specificity will depend on the intended objective of the analytical procedure.

1.5.2. ACCURACY

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or on an accepted reference value and the value found.

1.5.2.1. Assay

- Assay of Active Substance

- Assay of Medicinal products

Several methods are available to determine the accuracy.

a. Application of an analytical procedure to an analyte of known purity (e.g. reference material).

b. Comparison of the results of the proposed analytical procedure with those of a second well-characterized procedure, the accuracy of which is stated and/or defined (independent procedure)

c. Application of the analytical procedure to synthetic mixtures of the product components to which known quantities of the substance to be analyzed have been added.

1.5.2.2. Impurity (Quantification)

Accuracy should be assessed on sample (substance /products) spiked with known amounts of impurities. It should be clear how the individual or total impurities are to be determined.

E.g. Weight / Weight or area percent.

1.5.3. PRECISION

The precision of an analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision of an analytical procedure is usually expressed at the variance, standard deviation or coefficient of variation of a series of measurements.

Validation of tests for assay and for quantitative determination of impurities includes an investigation of precision.

1.5.3.1. REPEATABILITY

Express the precision under the same operating conditions over a short interval of time. Repeatability is also termed as intra - assay precision. It should be assessed using a minimum of nine determinations covering the specified range for the procedure (e.g. three concentration/three replicates each) or a minimum of determinations at 100% of the test concentration.

1.5.3.2. INTERMEDIATE PRECISION

The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used. The applicant should establish the effects of random events on the precision of the analytical procedure. Typical variations to be studied include days, analysts, equipment, etc.

1.5.3.3. REPRODUCIBILITY

Reproducibility is assessed by means of an inter-laboratory trial. Reproducibility should be considered in case of the standardization of an analytical procedure, for instance inclusion of procedures in Pharmacopoeias.

1.5.4. LINEARITY

Linearity of an analytical procedure is its ability (with in a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. If there is a linear relationship, test results should be evaluated by appropriate statistical methods. For example, calculation of a regression line by the method of least square. Therefore data from regression line itself may be helpful to provide mathematical estimates of the degree of linearity.

1.5.5. RANGE

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.

The following minimum specified ranges should be considered

- For the assay of an active substance or a finished product normally from 80-120 percent of the test concentration.

- For the content uniformity, covering a minimum of 70-130 percent of the test concentration.

- For dissolution testing, 20% over the specified range (e.g.), If the specifications for a controlled release product cover a region from 20% after 1 hour, upto 90% after 24 hours, the validated range would be 0-110% of label claim.

- For the determination of an impurity, the reporting level of an impurity to 120% of the specifications.

1.5.6. LIMIT OF DETECTION

The detection limit is determined by the analysis of samples with known concentration of analyte and by establishing that minimum level at which the analyte can reliably detected.

i. Based on visual evaluation

Visual evaluation may be used for non-instrumental methods but may be used with instrumental methods.

ii. Based on signal to noise ratio

Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio 3 or 2 : l is generally considered acceptable for estimating the detection limit.

iii. Based on the standard deviation of the response and the slope

The detection limit (DL) may be expressed as

$$D L = 3.3\sigma/S$$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope, S may be estimated from the calibration curve of the analyte. The estimate of σ may be carried out in a variety of ways.

a. Based on the Standard Deviation of the Blank

Measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.

b. Based on the calibration curve

A specific calibration curve should be studied using samples containing an analyte in the range of DL. The residual standard deviation of a regression line or the standard deviation of y-intercept of regression lines may be used as the standard deviation.

1.5.7. LIMIT OF QUANTIFICATION

The quantification limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision

i. Based on visual evaluation

Visual evaluation may be used for non-instrumental methods but may also be used with instrumental methods.

ii. Based on Signal- to-Noise ratio

Determination of the Signal-to-Noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and by establishing the minimum concentration at which the analyte can be reliably quantified. A typical Signal- to-Noise ratio is 10:1.

iii. Based on the Standard Deviation of the Response and the slope

The quantification limit (Q L) may be expressed as

$$QL = \frac{10\sigma}{S}$$

Where,

σ	=	the standard deviation of the response
S	=	the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte. The estimate may be carried out in a variety of ways including,

a. Based on standard deviation of the blank

Measurement of the magnitude of analytical background response is performed by an appropriate number of blank samples and calculating the standard deviation of these responses.

b. Based on the Calibration Curve

A specific calibration curve should be studied using samples containing an analyte in the range of QL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

1.5.8. ROBUSTNESS

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.

Examples of typical variations are

- Stability of analytical solutions.
- Extraction time.

Incase of liquid chromatography, examples of typical are

- Influence of variation of pH in a mobile phase
- Influence of variations in mobile phase composition,
- Different columns (different lots and / or suppliers)
- Temperature
- Flow rate

In the case of gas-chromatography, examples of typical variations are

- Different columns (different lots and/or suppliers),
- Temperature,
- Flow rate.

1.5.9. RUGGEDNESS

The United States pharmacopoeia (USP) define ruggedness as the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different labs, different analysis, different lots of reagents etc. Ruggedness is a measure of reproducibility of test results under normal expected operational conditions from laboratory to laboratory and from analyst to analyst.

1.6. SYSTEM SUITABILITY PARAMETERS

(Anonymous. USP, 1995; Sethi, 2001)

System suitability test are an integral part of Gas and Liquid Chromatography. They are used to verify that the resolution and reproducibility of the chromatographic system a are adequate for the analysis to be done. These 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.

There are numerous guidelines which detail the expected limits for typical chromatographic methods. In the current FDA guideline on "Validation of Chromatographic methods" the following acceptance limits are proposed as initial criteria.

i. Capacity factor (K')

It is the measure of a sample peak in the chromatogram being specific for a given compound, a parameter which specifies the delay of a substance to be separated.

$$K' = \frac{t_r - t_o}{t_o}$$

Where,

t_r = retention time measured from time of injection to time of elution of peak maximum.

t_o = void volume

Limit = generally the value of $K^{'}$ is 1-10.

ii. Resolution (R_s)

The resolution R_s is a function of column efficiency N and is specified to ensure that closely eluting compounds are resolved from each other to establish the general resolving power of the system and to ensure that internal standards are resolved from the drug.

$$R_s = \frac{t_2 - t_1}{0.5(w_1 - w_2)}$$

Where,

t_1 and t_2 = retention times of first and second adjacent bands.

Limit = R_s of >2 between the peak of interest and the closest potential interfering peak is desirable.

iii. Tailing factor (T)

The tailing factor T, a measure of peak symmetry, is unity for perfectly symmetrical peaks and its value increases as tailing becomes more pronounced.

In some cases, values less than unity may be observed. As peak asymmetry increases, integration, and hence precision becomes less reliable.

$$T = \frac{W_{0.05}}{2f}$$

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 baseline.

Limit: ≤ 2 is preferable.

iv. Theoretical plates (N)

The number of theoretical plates, N is a measure of column efficiency. For Gaussian peaks, it is calculated by the equations.

$$N = 16(t / w)^2 \text{ or}$$

$$N = 5.54(t / w_{1/2})^2$$

Where,

t = retention time of substance.

W = width of the peak at its base, obtained by extrapolating the relatively straight sides of the peak to the baseline.

$W_{1/2}$ = width of the peak at half height, obtained directly by electronic integrators.

The value of 'N' depends upon the substance being chromatographed as well as the operating conditions such as mobile phase, temperature etc.

Limit = N > 2000 is desirable.

1.7. PHARMACEUTICAL STATISTICS

(Sanford Bolton, 1990; Kenneth, 2001; Mendham, 1994)

Statistical techniques have been widely used in many diverse areas of scientific investigation. Statistical applications have been recognized as crucial to quality control procedure, test, specification and definitions. Principle of modern analytical techniques and skill in their application are necessary attribute of the successful pharmaceutical analyst, thus does not ensure the satisfactory solution of all the problem that may encountered. Some auxiliary knowledge methods that can aid the analyst in designing experiment, collecting data, and interpreting the result.

1.7.1. Linear regression

Linear regression a statistical technique that defines the functional relationship between two variable by best-fitting straight line. Once a linear relationship has been shown to have a high probability by the value of the correlation coefficient 'r', then the best straight line through the data points has to be estimated. This can often be done be done by visual inspection of the calibration graph, but in many cases it is far more sensible to evaluate the best straight line by linear regression (the method of least squares)

The equation of straight line is

$$y = mx + c$$

Where, y the dependent variable is plotted as result of changing x, the independent variable.

To obtain the regression line 'y on x' the slope 'm' of the line and the intercept 'c' on the y axis are given by the following equation.

$$m = \frac{N \Sigma xy - (\Sigma x)(\Sigma y)}{N \Sigma x^2 - (\Sigma x)^2}$$

and

$$c = \frac{(\Sigma y)(\Sigma x^2) - (\Sigma x)(\Sigma xy)}{N \Sigma x^{2-(}\Sigma x)^2}$$

1.7.2. Correlation coefficient (r)

It is a procedure commonly used to characterize quantitatively the relationship between variable. Correlation is related to linear regression. To establish whether there is a linear relationship between two variables x_1 and y_1, use Pearson's correlation coefficient r.

$$r = \frac{n \Sigma x_1 y_1 - \Sigma x_1 y_1}{\{[n \Sigma x_1^2 - (\Sigma x_1)^2][n \Sigma y_1^2 - (\Sigma y_1)^2]\}^{1/2}}$$

Where n is the number of data points.

The value of r must lie between +1 and -1, the nearer it is to +1, the greater the probability that a definite linear relationship exists between the variables x and y, values close to +1 indicate positive correlation and values close to -1 indicate negative correlation values of 'r' that tend towards zero indicate that x and y are not linearly related (they made be related in a non-linear fashion).

1.7.3. Standard deviation (SD)

It is commonly used in statistics as a measure of precision statistics as a measure of precision and is more meaningful than is the average deviation. It may

be thought of as a root-mean-square deviation of values from their average and is expressed mathematically as

$$S = \sqrt{\frac{\sum_{i=1}^{i=n}(x_i - \bar{x})}{N-1}}$$

Where,

S is standard deviation.

If N is large (50 or more) then of course it is immaterial whether the term in the denomination is N -1 or N

Σ = sum

$\bar{x}$ = Mean or arithmetic average.

$x - \bar{x}$ = deviation of a value from the mean

N = Number of observations

1.7.4. Percentage relative standard deviation (%RSD)

It is also known as coefficient of variation (CV). It is defined as the standard deviation (S.D) expressed as the percentage of mean.

$$CV \text{ or } \% \text{ RSD} = \frac{S.D}{\bar{x}} \times 100$$

Where,

S.D is the standard deviation,

$\bar{x}$ = Mean or arithmetic average.

The variance is defined as S^2 and is more important in statistics than S itself. However, the latter is much more commonly used with chemical data.

1.7.5. Standard error of mean (SE)

Standard error of mean can be defined as the value obtained by division of standard deviation by square root of number of observations. It is mathematically expressed as

$$S.E. = \frac{S.D.}{\sqrt{n}}$$

Where,

S.D = Standard deviation

n = number of observations.

Review of Literature

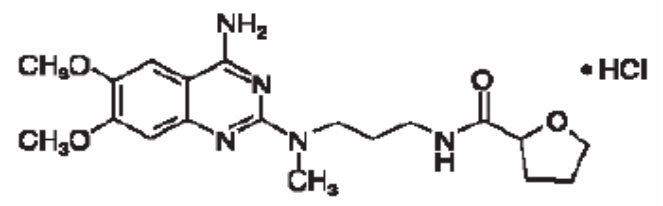
2. REVIEW OF LITERATURE

2.1. CHEMISTRY AND PHARMACOLOGY

2.1.1. CHEMISTRY OF ALFUZOSIN HC1

(Susan Budavani, 1998; Anthony Moffat, 1996)

i. STRUCTURE



ii. Chemical Name

(R,S)-N-[3-[(4-amino-6,7-dimethoxy-2-quinazolinyl) methylamino] propyl] tetrahydro-2-furancarboxamide hydrochloride.

iii. Molecular Formula

$C_{19}H_{27}N_5O_4$. HCl

iv. Molecular weight

425.9.

v. Description

Alfuzosin hydrochloride is a white to off-white crystalline powder

vi. Storage

Store below 25°C, protected from moisture.

vii. Solubility

It is freely soluble in water, sparingly soluble in alcohol, and practically insoluble in dichloromethane.

viii. Melting Point

Melting point of Alfuzosin HC1 was identified at 238° C

ix. Identification

Alfuzosin HC1 was identified and interpreted by IR spectrum which was given in Fig-1

2.1.2. PHARMACOLOGY OF ALFUZOSIN HC1

(Goodman and Gillman's, 2001; Parfitt, 1991; www.enwikepedia.com)

i. Mechanism of action

Alfuzosin HC1 is a quinazoline derivative which acts as a α_1-adrenergic blocking agent. It is structurally relative to prazosine which exhibits selectivity for α_1 adrenergic recptors in lower urinary tract. Blockade of these adrenoreceptors can cause relaxation of smooth muscle in bladder neck and prostate to relax resulting in an improvement of urine flow and a reduction in symptoms of benign prostatic hyperplasia(BPH).

ii. Pharmacokinetics

a. Absorption

The absolute bioavailability of Alfuzosin HC1 10 mg tablets under fed conditions is 49%. Following multiple dosing of 10 mg Alfuzosin HC1 under fed condition, the time to maximum concentration is 8 hours.

b. Distribution

The volume of distribution following intravenous administration in healthy male middle-aged volunteers was 3.2 L/kg. Results of in vitro studies indicate that Alfuzosin is moderately bound to human plasma proteins (82% to 90%), with linear binding over a wide concentration range (5 to 5,000 ng/ml).

c. Metabolism

Alfuzosin HC1 undergoes extensive metabolism by the liver, with only 11 % of the administered dose excreted unchanged in the urine. Alfuzosin is metabolized by three metabolic pathways: oxidation, O-demethylation and N-dealkylation. The metabolites are not pharmacologically active. CYP3A4 is the principal hepatic enzyme isoform involved in its metabolism.

d. Elimination

Following oral administration of Alfuzosin HC1 solution, the recovery of radioactivity after 7 days (expressed as a percentage of the administered dose) was 69% in feces and 24% in urine. Following oral administration of Alfuzosin 10 mg tablets, the apparent elimination half-life is 10 hours.

iii. Contra indication

Alfuzosin HC1 should not be used in patients with moderate or severe hepatic insufficiency, since Alfuzosin blood levels are increased in these patients. Alfuzosin should not be co-administered with potent CYP3A4 inhibitors such as ketoconazole, itraconazole, and ritonavir, since Alfuzosin blood levels are increased. Alfuzosin HC1 is contraindicated in patients known to be hypersensitive to Alfuzosin HC1.

iv. Drug Interactions

CYP3A4 is the principal hepatic enzyme isoform involved in the metabolism of Alfuzosin. Repeated administration of 400 mg of ketoconazole, a potent inhibitor of CYP3A4, increased Alfuzosin C_{max} 2.3-fold and AUC_{last} 3.2-fold following a single 10 mg dose of Alfuzosin. Therefore Alfuzosin should not be co-administered with potent inhibitors of CYP3A4 because exposure is increased, (e.g., ketoconazole, itraconazole, or ritonavir).

v. Side effects

Body as a whole*:* pain

Gastrointestinal system*:* abdominal pain, dyspepsia, constipation, nausea

Reproductive system: impotence

Respiratory system*:* bronchitis, sinusitis, pharyngitis.

vi. Dosage and Administration

The recommended dosage is one 10mg Alfuzosin HCl (extended-release tablets) tablet daily to be taken immediately after the same meal each day. The tablets should not be chewed or crushed.

vii. Clinical use

Alfuzosin HC1 is indicated for the treatment of the sign and symptoms of benign prostatic hyperplasia. Alfuzosin HC1 is not indicated for the treatment of hypertension.

2.2. REPORTED ANALYTICAL METHODS FOR ALFUZOSIN HC1

2.2.1. Wiesner J L et al., (2003), Selective, sensitive and rapid liquid chromatography tandem mass spectrometry method for the determination of Alfuzosin in human plasma.

A selective, sensitive and rapid liquid chromatography-tandem mass spectrometry method for the determination of alfuzosin in plasma was developed. A PE Sciex API 2,000 triple quadrupole mass spectrometer in multiple reaction monitoring (MRM) mode, using TurboIonSpray with positive ionization was used. Using prazosin as an internal standard, liquid-liquid extraction was followed by C_{18} reversed-phase liquid chromatography and tandem mass spectrometry. The mean recovery for alfuzosin was 82.9% with a lower limit of quantification set at 0.298 ng/ml, the calibration range being between 0.298 and 38.1 ng/ml. This assay method makes use of the increased sensitivity and selectivity of tandem mass spectrometric (MS-MS) detection to allow for a more rapid (extraction and chromatography) and selective method for the determination of alfuzosin in human plasma.

2.2.2. Wei X et al., (2007), On-line solid-phase extraction with a monolithic weak cation-exchange column and simultaneous screening of α-adrenergic receptor antagonist in human plasma.

An on-line SPE-HPLC method, using a monolithic poly(glycidyl methacrylate-co-ethylene glycol dimethacrylate) (poly(GMA-EDMA)) based weak cation-exchange (WCX) column, was developed for simultaneous determination of alpha1-adrenergic receptor antagonists in human plasma. The monolithic WCX column was prepared by an in-situ polymerization protocol and modified stepwise with ethylenediamine and chloroacetic acid. On connecting this column to an

injection valve, an on-line SPE protocol could be established for removal of matrices (mainly proteins and lipids) and preconcentration of four α_1-adrenergic receptor antagonists in human plasma. This method was validated and then used for determination of terazosin, prazosin, doxazosin and Alfuzosin in clinical plasma samples.

2.2.3. Chandra A et al., (2007), Development and Validation of RP-HPLC method for the estimation of Alfuzosin HC1 in bulk and Tablets.

A RP- HPLC method has been developed for the estimation of Alfuzosin HC1 in Bulk and Pharmaceutical dosage form. The quantification was carried out using a RP stainless steel column in isocratic mode, with mobile phase consisting of methanol, water, triethyl amine in the ratio of 60 : 39.6 : 0.4 (v/v) and added concentrated formic acid to make up to the p^H-4 . The mobile phase was pumped at a rate of 0.9 ml/min and the detection was carried out at 245 nm and the linearity was found to be in the range of 10 to 1000 µg/ml. The proposed method was found to be simple, precise, accurate, rapid and reproducible for the estimation of Alfuzosin HC1 in bulk and tablets.

2.2.4. British Pharmacopoeia-2003, Vol.1, The Department of Health, London.

BP recommends non-aqueous titration for estimation of Alfuzosin HC1 in bulk drug. In this method drug was titrated with 0.1 M perchloric acid and end point is determined by potentiometrically.

2.2.5. Rouchouse A et al., (1990), Direct High-performance liquid chromatographic determination of the enantiomers of Alfuzosin in plasma on a second generation α_1 -acid glycoprotein chiral stationary phase.

A direct liquid chromatographic method was developed for the determination of the enantiomers of alfuzosin in human plasma, without derivatization, on a chiral α_1 -acid glycoprotein column. The influence of pH, of uncharged organic solvents and of a cationic modifier (tetrabutylammonium) of the mobile phase on retention and enantioselectivity was evaluated. The enantiomers and an internal standard, structurally related to alfuzosin, were extracted from plasma with dichloromethane-diethyl ether from alkaline solution, then separated with a mobile phase of 0.025 M phosphate buffer (pH 7.4) containing 0.025 M tetrabutylammonium bromide-acetonitrile (94:6, v/v). The limit of quantification for each isomer was 1 ng/ml. The method has been applied to the determination of the pharmacokinetic profile of Alfuzosin HC1 enantiomers in healthy volunteers after intravenous administration of the racemate.

2.2.6. Giuseppe Carlucci et al., (2006),Determination of Alfuzosin HC1 in human Plasma by High-Performance Liquid Chromatography with Column-Switching.

A high - performance liquid chromatographic method for the determination of Alfuzosin HC1 in human plasma has been developed and validated. A column-switching procedure without extraction was used to isolate the drug from biological matrix prior to the quantitative analysis. The lower limit of detection for the analyte was 1 ng/ml. The method was linear from 2 to 150 ng/ml for human plasma. Within- and between-assay precision and accuracy were all found to be <5.2% at the eight concentrations evaluated.

2.2.7. Salah Fayed A et al., (2006), Validated HPLC and HPTLC stability-indicating methods for determination of Alfuzosin HC1 in bulk powder and pharmaceutical formulations.

Two sensitive, selective, and precise stability-indicating, high-performance liquid chromatography and high-performance thin-layer chromatography methods have been developed for the determination of Alfuzosin HC1 in the presence of its degradation products. Alfuzosin HC1 was subjected to stress alkaline, acidic, oxidative, thermal, and photo-degradation. The drug could be well separated from the degradation products upon applying the two methods. Separation by HPLC was achieved using an Xterra RP18 column and acetonitrile/0.02 M KH_2Po_4 (pH=3) in a ratio of 20: 80 as mobile phase. The flow rate was 1 ml/min. The linearity range was 0.25 to 11 µg/ml with mean percentage recovery of 100.26 +/- 1.54.

The HPTLC method used ALUGRAM Nano-SIL silica gel 60 F_{254} plates; the optimized mobile phase was methanol/ammonia (100:1.2). Quantitatively the spots were scanned densitometrically at 245 nm. A second order polynomial equation was used for the regression. The range was 0.5-7 microg/spot. The mean percentage recovery was 100.13 +/- 1.67.

Aim & Plan of Work

3. AIM AND PLAN OF WORK

The drug analysis plays an important role in the development, manufacture and therapeutic use of drugs. Most of the pharmaceutical industries do the quantitative chemical analysis to ensure that the raw material used and the final product thus obtained meet certain specification and to determine how much of each components are present in the final product.

Extensive literature survey reveals that Afuzosin HC1 is a official drug of BP 2003 which recommends non-aqueous titration for estimation of Alfuzosin Hcl in bulk drug only where the end point is determined by potentiometry. The titrimetric method has its own limitation is that; it is very difficult to measure small quantity of drug sample and less sensitive. Further, literature review revealed, HPLC and HPTLC methods have been reported in biological fluids, bulk drug and pharmaceutical dosage form, but UV-Spectrophotometric method was not reported. Hence, an attempt is made in this project to develop simple, cost effective, sensitive, precise and accurate spectrophotometric methods HPLC for Alfuzosin HC1.

Plan of the developed experimental protocol.

- Selection, Collection and Identification of Alfuzosin HC1 for analysis.
- Development of simple, cost effective and accurate spectrophotometric methods.
- Development of a rapid and accurate RP-HPLC method using UV detection.
- Analysis of marketed formulations of Alfuzosin HC1.
- Validation of proposed methods.
- Statistical analysis of developed analytical methods.

Materials & Methods

4. MATERIALS AND METHODS

4.1. MATERIALS

4.1.1 DRUG SAMPLE AND FORMULATIONS

Afuzosin HC1 was generously gifted by Aurobindo Pharma Ltd., Hyderabad.

The following three formulations containing 10 mg were selected for the study and procured from local pharmacy.

1. Alfoo (F_1) - Dr. Reddy's Laboratories, Hyderabad.
2. Alfusin (F_2) - Cipla Pharmaceutical Pvt Limited, Mumbai.
3. Xelflo (F_3) - Sun Pharma, Mumbai.

4.1.2. REAGENTS AND CHEMICALS

All the chemicals used were of analytical and HPLC grade and procured from Qualigens India Ltd., Mumbai, Loba Chemicals Ltd., Mumbai and Ranbaxy Fine Chemicals Ltd., Mumbai. The chemicals used for the study are as follows,

S.No.	Chemicals / Solvents used	Grade
1	Sodium Hydroxide	Analytical Grade
2	Chloroform	Analytical Grade
3	Hydrochloric Acid	Analytical Grade
4	Methanol	HPLC Grade
5	Water	HPLC Grade & Analytical Grade
6	Ortho phosphoric Acid	Analytical Grade
7	Bromocresol green	Analytical Grade
8	Ethanol	Analytical Grade
9	Potassim dihydrogen ortho phosphosphate	Analytical Grade
10	Potassium Hydrogen phthalate	Analytical Grade

4.1.2.1. Preparation of reagents

a. Acid phthalate buffer solution p^H-3.0 (Anonymous. The Indian Pharmacopoeia, 1996)

Place 50 ml of the 0.2 M potassium hydrogen phthalate solution in a 200 ml volumetric flask, add 22.3 ml of the 0.2 M HC1 solution and then add water to volume and p^H was checked with p^H meter.

b. 0.2 M Potassium Hydrogen Phthalate

Dissolved 40.846 gm of potassium hydrogen phthalate in water and dilute with water to 1000 ml.

c. Bromo Cresol Green solution

Dissolved 50 mg of the Bromo cresol green in 0.72 ml of 0.1 M sodium hydroxide and 20 ml of ethanol (95%). After solution is effected add a sufficient quantity of water to produce 100 ml.

d. 0.1 M Sodium Hydroxide

4.2 gm of sodium hydroxide in sufficient CO_2 free water to produce 1000 ml.

e. 1 M HC1

85 ml of HC1 was diluted with water to produce 1000 ml.

f. 0.2 M Hydrochloric acid

HC1 was diluted with water to contain 7.292 gm of HC1 in 1000 ml.

g. 10 mM Phosphate Buffer solution(p^H-3)

1.360 gm of potassium dihydrogen ortho phosphate was diluted with water (HPLC Grade) to 1000 ml. The p^H-3 was adjusted with ortho phosphoric acid and checked with p^H meter

4.1.3 INSTRUMENTS

Instruments employed for the study were,

A) SHIMADZU AX-200 Digital balance

B) SHIMAZU-1700 Double beam UV-VISIBLE Spectrophotometer with pair of 10 mm matched Quartz cells

C) HPLC

- SPD-10Avp SHIMADZU UV-Visible detector
- LC- 10ATVP SHIMADZU solvent deliver module

D) ELICO SL 159 UV –VIS Spectrophotometer

E) ELICO pH METER

Instruments Specifications

A) Shimadzu AX – 200 digital balance: (Anonymous.Shimadzu Instruction Manual)

Specifications	
Weighing capacity	200gms
Minimum display	0.1mg
Standard deviation	≤ 0.1mg
Operation temperature range	5 to 40°C

B) Shimadzu UV – Visible spectrophotometer: (Anonymous.Shimadzu Instruction Manual)

Model: Shimadzu, UV-1700; Cuvetts: 1cm quartz cells.

Specifications	
Light source	20 W halogen lamp, Deuterium lamp. Light source position automatic adjustment mechanism.
Monochromator	Aberration-correcting concave holographic grating
Detector	Silicon Photodiode
Stray Light	0.04% or less (220 nm: NaI 10g/l) 0.04% or less (340 nm: $NaNO_2$ 50g/l)
Measurement wavelength range	190~1100 nm
Spectral Band Width	1nm or less (190 to 900 nm)
Wavelength Accuracy	±0.5nm automatic wavelength calibration mechanism
Recording range	Absorbance : - 3.99~3.99Abs Transmittance : - 399~399%
Photometric Accuracy	±0.004Abs (at 1.0 Abs), ±0.002 Abs (at 0.5 Abs)
Operating Temperature/Humidity	Temperature range : 15 to 35°C Humidity range : 35 to 80% (15 to below 30°C) 35 to 70% (30 to 35°C)

C) Shimadzu High Performance Liquid Chromatography: (Anonymous.Shimadzu Instruction Manual)

Detector Specifications	
Light source	Deuterium Arc lamp
Measurement wavelength range	190 to 700 nm
Spectral Band Width	5 nm
Wavelength Accuracy	± 1 nm
Cell path length	10 nm
Cell volume	20µl
Operating temperature range	4 to 35°C (39 to 104°F)
Recording range	0.0001 to 4.000AUFS
Operating temperature/Humidity	40 to 75%

Pump Specifications	
Pump type	Double reciprocating plunger pump
Pumping methods	Constant flow delivery and constant pressure
Suction filter	45 µm
Line filter	5 µm mesh
Operating temperature	4 to 35°C (39 to 104°F)

D) pH-Meter

Make	:	Elico LI 120
Electrode	:	Deep vision
pH range	:	0-14
Electrode temperature range	:	20-100˚C

4.2. METHODS

The analysis of Alfuzosin HC1 was done by following methods:

4.2.1. UV-Spectroscopic method

4.2.2. Visible Spectrophotometric method

4.2.3. High Performance Liquid Chromatographic method

4.2.1. UV SPECTROSCOPIC METHOD

4.2.1.1 Selection of solvent and λ_{max}

The solubility of Alfuzosin HC1 was determined in a variety of solvents as per Indian pharmacopeia 1996. Solubility test for Alfuzosin HC1 in non polar and polar solvent by increasing dielectric constant was carried out to know solubility and choose solvent for the proposed method. From the solubility studies, 0.1M NaOH was selected as solvent. The drug solution was prepared and scanned from 200- 400 nm. and shows constant λ_{max} at 250 nm and 350 nm. The stability of the absorbance of drug solution at these λ_{max} was checked. It was stable only at λ_{max} 350 nm, which was taken for the proposed method. Alfuzosin HC1 was stable for 180 mins.

4.2.1.2. Preparation of standard solution

An accurately weighed quantity of 25 mg of pure Alfuzosin was dissolved in a minimum quantity of 0.1 M NaOH in a 100 ml volumetric flask and the total volume was brought to 100 ml with 0.1 M NaOH (250 μg /ml).

4.2.1.3. Linearity and calibration

Into a series of five 25 ml volumetric flask, Aliquots from stock solution (1, 1.5, 2, 2.5 & 3ml) were taken and made up to the mark with 0.1M NaOH. The absorbance was measured at 350 nm against 0.1 M NaOH as blank. The calibration curve was plotted in the concentration range of 10-30 μg/ml.

4.2.1.4. Quantification in formulation

4.2.1.4.1. Preparation of Test solution

Twenty tablets of each formulation F1, F2 & F3 (Alfoo, Anfusin and Xelflo) containing 10 mg of Alfuzosin HCl were accurately weighed and powdered. Powered tablet equivalent to 25 mg of Alfuzosin HC1 was transferred into 250 ml volumetric flask, added 50 ml of 0.1 M NaOH and sonicated for five minutes and repeated the sonication consequently by four time (4×50 ml) to produce 250 ml. The solution was filtered through Whatmann filter paper No.41 (100 μg/ml).

4.2.1.4.2. Assay procedure

From above clear solution, transferred 5 ml to a 25 ml standard flask and made up to the mark to produce the concentration of 20 μg/ml. The absorbance measurements were made six times for each formulation. The amount of Alfuzosin was calculated from the respective calibration curve.

4.2.1.5. Recovery studies

From the pre analyzed formulation, a known quantity of standard solution was added and the contents were mixed well, finally made up the volume with 0.1M NaOH. Absorbance was measured at its λ_{max}. Amount present was calculated from slope and intercept. Then the recovery studies were determined by using the following formula.

$$\% \text{ of Recovery} = \frac{N\Sigma xy - \Sigma x \ \Sigma y}{N\Sigma x^2 - (\Sigma x)^2} \times 100$$

Where,

N = Number of observations

X = Amount added in μg/ml

Y = Amount recovered in μg /ml

4.2.1.6. Limit of Detection (LOD) and Limit of Quantification (LOQ)

Preparation of calibration curve from the serial dilutions of standard was repeated for six times. The limit of detection and limit of quantification was calculated by using the average value of slope and standard deviation.

4.2.1.7. Repeatability

Repeatability was done by inter day and intra day precision. The assay procedure were repeated three times on the same day and one time each on three successive days.

4.2.2. VISIBLE SPECTROPHOTOMETRIC METHOD

4.2.2.1. Selection of Colorimetric Studies (Sethi, 2003)

Amines in their protonated state form a complex with an anionic species (acidic dyes) to form neutral ion-pair complexes which are distinguished by their solubility in organic solvents such as chloroform, benzene, dichloromethane etc. Thus by using a pairing agent, a colored complex is produced and the amines can thus be measured colorimetrically. The equilibrium expression for ion-pair formulation can be expressed as

$$A^+_{aq} + D^-_{aq} \longrightarrow AD_{0rg}$$

$\mathbf{A^+_{aq}}$ is protonated amine in aqueous phase, $\mathbf{D^-_{aq}}$ **is** the anionic pairing dye in aqueous phase and $\mathbf{AD_{org}}$ is the final ion pair complex method has been extensively used in this text for estimation of basic nitrogenous substances employing several anionic pairing ions (dyes).

The compound Alfuzosin HC1 contains tertiary amino group in its side chain which was readily complexed with an anionic species (dye) using an ion pairing agent in chloroform and the neutral pair complex produced was measured calorimetrically**.** In order to develop a method in visible spectroscopy, its structure-side chain p^{Ka} value, p^H and solubility were studied. Hence the above principle was highly applicable for the drug Alfuzosin HC1.

4.2.2.2. Selection of solvent

From the solubility table, it was found that the drug of Alfuzosin HC1 was soluble in the organic solvent, chloroform. Under acidic conditions, the compound Alfuzosin HC1 containing tertiary amino group which readily complexes with bromo cresol green (acitic dye) to produce yellow colored complex which was extracted with chloroform. The chloroform extract was scanned from 400- 800 nm. From the spectra, λ_{max} was found to be 416 nm. Alfuzosin HC1 was stable for 90 mins in chloroform.

4.2.2.3. Preparation of Standard Solution

About 10 mg of accurately weighed quantity of Alfuzosin HC1 was dissolved in 50 ml of distilled water and the total volume was brought to 100 ml with water (100 µg/ml).

4.2.2.4. Optimization of p^H

The absorbance of Alfuzosin HC1 in different p^H of acid phthalate buffer (p^H-2.4, p^H-2.6, p^H-2.8, p^H-3.0 & p^H-3.2) was observed. The p^H of the buffer was optimized by steady in absorbance. It was found that the absorbance of Alfuzosin in buffer p^H-3 was same up to 90 mins.

4.2.2.5. Optimization of Dye solution

The volume of Bromocresol green was optimized by taking 5 ml of standard solution of drug in six separating funnel in which 10 ml of acid phthalate buffer (p^H-3) and the bromocresol green(dye) 5, 10, 15, 20, 25 and 30 ml were added. Then coloured complex was extracted with chloroform. The absorbance of chloroform extract of each solution was measured at 416 nm against chloroform as blank. It was found that, 20 ml was the optimized volume of dye with the above optimized condition.

4.2.2.6. Linearity and calibration

From the standard solution 1-5 ml were taken, separately into a separating funnel, added 20 ml of dye solution and 2 drops of 1M HC1 to obtained yellow colour complex, Then, added 10 ml buffer solution and extracted the yellow colour complex with 20, 15 &15 ml of chloroform. The chloroform layers were combined and diluted to 50 ml with chloroform. The absorbance was measured at 416 nm against chloroform blank. The calibration curve was plotted in the concentration range of 2-10 μg/ml.

4.2.2.7. Quantification in formulations

i. Preparation of Test solution

Twenty tablets of each formulations F1, F2 & F3 (Alfoo, Anfusin and Xelflo) containing 10 mg of Alfuzosin HC1 were weighed accurately and powered. Powdered tablet equivalent to 25 mg of Alfuzosin HC1 was transferred into 250 ml volumetric flask, added 50 ml of 0.1M NaOH. It was sonicated for five minutes and repeated the sonication with distilled water consequently by four times (4×50 ml) to produce 250 ml. The solution was filtered through Whatmann filter paper No.41 (100 µg/ml).

ii. Assay Procedure

From the above clear solution, pipetted out 25 ml to a 50 ml standard flask and made upto the mark with distilled water to produce 50 µg/ml. From this, 6 ml of solution was transferred to a separating funnel, added 20 ml of dye solution and 2 drops of 1M HC1 to obtain yellow color. Then, added 10 ml of acid phthalate buffer solution and extracted the yellow colour drug complex with 20, 15 & 15 ml of chloroform. Combined the chloroform and obtained the concentration of 6 µg/ml. The above procedure was repeated for six times for each formulation. The amount of Alfuzosin was calculated from the respective calibration curve.

4.2.2.8. Recovery

To the pre analyzed solution of formulation, a known quantity of standard solution was added and the contents were mixed well, finally made up to volume with distilled water. Absorbance was measured at its λ_{max}. Amount present was calculated from slope and intercept and the recovery studies were calculated.

4.2.2.9. Limit of Detection (LOD) and Limit of Quantification (LOQ)

Preparation of calibration curve from the serial dilutions of standard was repeated for six times. The limit of detection and limit of quantification was calculated by using the average value of slope and standard deviation.

4.2.2.10. Repeatability

Repeatability was done by inter day and intra day precision. The assay procedure was repeated three times on the same day and one time each on three successive days.

4.2.3. HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD

4.2.3.1. Selection of chromatographic method

Proper selection of the method depends upon the nature of the sample, molecular weight, and solubility. The drug selected for the present study was polar. So reverse phase chromatography can be used. This RP-HPLC was selected for the initial separation because of its simplicity and suitability from the literature and with the knowledge of properties, C_{18} column was chosen as stationary phase with different composition of methanol and buffer were used.

4.2.3.2. Selection of λ_{max} for detection of compound

A solution of Alfuzosin HC1 (10 µg/ml) was scanned in UV region using the solvent, methanol: buffer (p^H-3) in the ratio of 80:20. The λ_{max} was found at 245 nm. This wavelength was used for the estimation of Alfuzosin HC1 by HPLC method.

4.2.3.3. Initial separation conditions

Following chromatographic conditions were fixed initially to improve the separation of drug,

Stationary phase - C_{18} Column (150 mm X 4.5mm i.d., 5 µm)

Mobile phase - Methanol: Buffer

Ratio - 80 : 20

Detection wavelength - 245 nm

Flow rate - 1 ml/min

Sensitivity - 0.01 AUFS

Operating pressure - 160 Kgf/cm^2

Temperature - Room temperature

4.2.3.4. Effect of ratio of mobile phase

Under the said conditions, different ratios of mobile phase were attempted using methanol: buffer (80:20, 70:30, 60:40 and 50:50). Chromatograms was recorded for all the above ratios, system suitability parameters were calculated.

It was seen that, while increasing the buffer ratio, optimum parameters results were obtained and the peak was sharp, hence the ratio of 50: 50 was selected for further studies.

4.2.3.5. Optimized chromatographic conditions

Following optimized chromatographic parameter were used for the estimation of Alfuzosin HC1

Mode of operation - Isocratic

Stationary phase - C_{18} Column (150 mm X 4.5mm i.d., 5 µm)

Mobile phase - Methanol : Phosphate Buffer (p^H-3)

Ratio - 50 : 50

Detection wavelength - 245nm

Flow rate - 1.0 ml/min

Temperature - Ambient

Sample volume - 20 µl

4.2.3.6. Preparation of standard Alfuzosin HC1 solution

An accurately weighed quantity of 50 mg of alfuzosin HC1 was dissolved in a minimum quantity of mobile phase in 50 ml volumetric flask. The total volume was brought to 50 ml with more mobile phase (1000 µg/ml).

4.2.3.7. Linearity and calibration

From the standard solution 1 ml was transferred into a 50 ml standard flask and made up to the mark with mobile phase to produce 20 µg /ml. To the series of five 10 ml standard flask, added 0.5 to 2.5 ml of above solution and made up to the mark to obtain the concentration range from 1 to 5 µg/ml and the calibration curve was plotted between concentration and peak area.

4.2.3.8. Quantification in formulations

i. Preparation of Alfuzosin HC1 test solution

Twenty tablets of F_1 formulation (Alfoo) containing 10 mg of Alfuzosin HC1 was weighed accurately and powered. Powdered tablet equivalent to 20 mg of Alfuzosin HC1 was transferred into 100 ml volumetric flask. To this, 50 ml of methanol was added and sonicated for 15 minutes. Then it was made up to the volume with methanol (20 µg/ml). The solution was filtered through membrane filter (0.45 µm).

ii. Assay procedure

From the above clear solution, pipetted out 5 ml to a 50 ml standard flask and made up to the mark with mobile phase to produce 20 µg/ml. The 1.5ml of above solution was transferred into a 10 ml volumetric flask to produce 3 µg/ml solutions. The peak area measurements were done by injecting the sample (3 µg/ml) six times and the amount of Alfuzosin calculated from the respective calibration curve.

4.2.3.9. Recovery experiments

To ensure the reliability of the method, recovery studies were carried out by mixing a known quantity of standard drug with the pre analyzed by the proposed method. The percentage of recovery was calculated.

4.2.3.10. System Suitability Studies

The system suitability studies were carried out as specified in ICH guidelines and USP. The parameters like, Tailing factor, Asymmetric factor, Theoretical plate and Capacity factor were calculated.

Results & Discussion

5. RESULTS AND DISCUSSION

UV-VISIBLE SPECTROSCOPIC METHODS

To ensure the percentage purity of Alfuzosin HC1 in various formulations, the UV-Visible spectroscopic methods were developed. The methods were very simple, economic and applicable for quantification of drug in bulk and in formulations.

5.1. UV-Spectroscopic method

The solubility of Alfuzosin HC1 was determined in a variety of solvents as per IP 1996. The solvents ranging from non-polar to polar were used. The solvents used were water, 0.1M HC1, 0.1M NaOH, methanol, acetonitrile, acetone, chloroform, diethyl ether, dichloroethan, ethyl acetate, dimethyl formamide and ethanol. To the drug (10 mg) in the test tube, added solvent slowly, initially solubility was checked in water, later proceed to other solvents. The solubility profile of Alfuzosin HC1 was given in Table- 1

From solubility studies, water and 0.1M sodium hydroxide was chosen as a solvent for the proposed method. But due to the instability of Alfuzosin HC1 in water, only 0.1 M NaOH was used for UV-spectroscopic studies of the drug in bulk and its formulation.

Drug was dissolved in 0.1M NaOH and made further dilutions with 0.1M NaOH to produce 15 µg/ml. It was scanned in the range of 200 - 400 nm and shows constant λ_{max} at 250 nm and 350 nm. This is shown in Fig.2. The stability of the absorbance of drug solution at these λ_{max} was checked. It was stable only at λ_{max} 350 nm, which was taken for the proposed method.

The linearity of the Alfuzosin HC1 was found from its calibration curve which was shown in Fig-3. The optical characteristic such as beers law limits (10 - 30 µg/ml), molar extinction co-efficient (5545.056), sandell's sensitivity (0.07485), correlation coefficient (0.999914), slope (0.01336) and intercept (0.0088) were calculated and were shown in Table- 3.

The limit of detection and the limit of quantification were determined from the linearity studies which have been done six times and then LOD and LOQ was calculated by using slope and standard deviation. The limit of detection was found to be 2.57 µg/ml and the limit of quantification was found to be 7.79 µg/ml. They have been shown in Table -3.

From the linearity curve, the middle concentration of 20 µg/ml was selected and quantification of drug in formulation was performed. The formulations of Alfoo, Alfusin and Xelflo were selected for analysis. The amount present was determined by average of six replicate analyses and it was found to be 9.81 mg, 9.96 mg and 10.00 mg respectively. The results were shown in Table -3 to 5.

To evaluate the accuracy of the proposed method, a known amount of pure drugs were added to the previously analyzed solution containing pharmaceutical formulations and their recoveries were calculated. The percentage recovery of Alfoo, Alfusin and Xelflo was 100.05%, 100.81% and 99.49% respectively. These values were given in Table- 6 to 8.

Precision of the proposed method has been done by inter day and intraday analysis of formulation. The assay procedure was repeated three times on the same day and one time each three successive days. The percentage standard deviation for interday and intraday analysis of formulations was found and given in the Table-9-11.

5.2. Visible spectroscopic method

The drug was dissolved in distilled water and diluted to prepare require concentration of 25 µg/ml. The compound Alfuzosin HC1 containing tertiary amino group which readily complexed with bromocresol green dye and produced yellow colour complex under acetic condition. The coloured drug complex was extracted with chloroform and produced a concentration of 2.5 µg/ml. It was scanned in the range of 250 - 600 nm and it shows the constant λ_{max} at 416 nm. This is shown in Fig-4. Stability of absorption at their λ_{max} was also checked.

The linearity of the Alfuzosin HC1 was found from its calibration curve which was shown in Fig-5. The optical characteristics such as beers law limits (2–10 µg/ml), molar extinction co-efficient (23319.18), sandell's sensitivity (0.016706348), correlation coefficient(0.99958), slope(0.05986) and intercept (0.0002063) were calculated and shown in Table- 12. The results were found to be satisfactory.

The limit of detection and the limit of quantification were determined from the linearity studies which have been done six times and then LOD and LOQ was calculated by using slope and standard deviation. The limit of detection was found to be 0.180706281µg/ml and the limit of quantification was found to be 0.547594 µg/ml. They have been shown in Table – 12.

From the linearity curve, the middle concentration of 6 µg/ml was selected and quantification of Alfuzosin HC1 in formulations were performed. The formulations of Alfoo, Alfusin and Xelflo were selected for analysis and the amount present was found to be 9.92 mg, 10.00 mg and 9.84 mg respectively. The results were shown in Table - 13 to15.

To evaluate the accuracy of the proposed method, a known amount of pure drugs were added to the previously analyzed solution containing pharmaceutical formulation and the recoveries were calculated. The percentage recovery of Alfoo, Alfusin and Xelflo was 99.16 %, 99.82% and 99.50% respectively. The values were given in Table -16 to18.

Precision of the proposed method has been done by inter day and intraday analysis of formulation. The assay procedure was repeated three times on the same day and one time each three successive days. The percentage standard deviation for interday and intraday analysis of formulations was found and given in the Table – 19 to 21

5.3. RP - HPLC METHOD

An effort has been made to identify a simple, cost effective, economic, specific and accurate method for the estimation of Alfuzosin HC1 in raw material and formulations.

The λ_{max} of Alfuzosin HC1 in mobile phase was found at 245 nm. The four different combinations of mobile phase were employed for the optimization of HPLC analysis. A mobile phase consisting of methanol : 10 mM phosphate buffer-(pH 3) in ratio of 80 : 20, 70 : 30, 60 : 40 and 50 : 50 was used and chromatogram were recorded at the flow rate of 1.0 ml/min, which were shown in Fig- 6 to 9 respectively.

Numbers of peaks were produced and the system suitability parameters were not with in the limits in the ratio of 80 : 20, 70 : 30 and 60 : 40 of mobile phase. So these three ratio of mobile phases were not selected for HPLC studies. But the parameters were within the limit in 50: 50 ratio of mobile phase.

Concentrations of raw material such as 1-5 µg/ml of Alfuzosin HC1 were injected and the chromatograms were recorded. They were shown in the Fig. 10-14 and the calibration curve for the same concentrations was plotted against peak area and shown in Fig. 15. The optical characteristics such as beers law limits (1-5µg/ml), molar extinction co-efficient (6.22363E+11), sandell's sensitivity (6.82194E - 10), correlation coefficient (0.9995), slope (1465781.9) and intercept (– 45649.76) were calculated and shown in Table-23. The results were found to be satisfactory.

The system suitability test parameters such as Theoretical plates (3090), Tailing factor (1.45), Asymmetric factor (1.71) and Capacity factor (0.96) were calculated for a mean concentration of 3 µg/ml and were shown in the Table- 22. The parameters were found to be satisfactory as per USP guidelines. Injection repeatability was performed for same concentrations six times to the formulation (Alfoo) and the amount present was found to be 9.91 mg. The values were shown in Table-24. Hence, precision was confirmed by repeatable injection of the formulation and chromatograms were shown in the Fig-16 to 21.

Accuracy was confirmed by recovery studies by adding known amount of pure drug to the previously analyzed formulation and the mixture was analyzed by the proposed method and chromatograms were shown in the Fig-22 to 27. The percentage recovery of Alfoo was found to be 99.86 %. The values were given in the Table-25. The results indicated that the excipients and additives did not interfer in the developed method.

All the above parameters combined with the simplicity and ease of operation ensures that the proposed method may be applied for the estimation of Alfuzosin HC1 in raw material and pharmaceutical dosage forms.

Summary & Conclusion

6. SUMMARY AND CONCLUSION

The proposed analytical methods are simple, economical, rapid, sensitive, reproducible and accurate for the estimation of Alfuzosin HC1. The method adopted for our studies are,

1. UV spectroscopic method
2. Visible spectroscopic method and
3. RP-HPLC method.

- The drug samples were analyzed by UV spectroscopy method using 0.1M NaOH as solvent at λ_{max} 350 nm and the content of drug present in each formulation was found to be,
  - Alfoo - 9.81 mg (98.10%)
  - Alfusin - 9.96 mg (99.60%)
  - Xelflo - 10.00 mg (100.00%)
- The drug samples were also analysed by visible spectroscopy by using Ion-pair complex method. The λ_{max} was found at 416 nm. The content of drug present in each formulation was found to be,
  - Alfoo - 9.92 mg (99.20%)
  - Alfusin - 10.00 mg (100.00%)
  - Xelflo - 9.84 mg (98.40%)

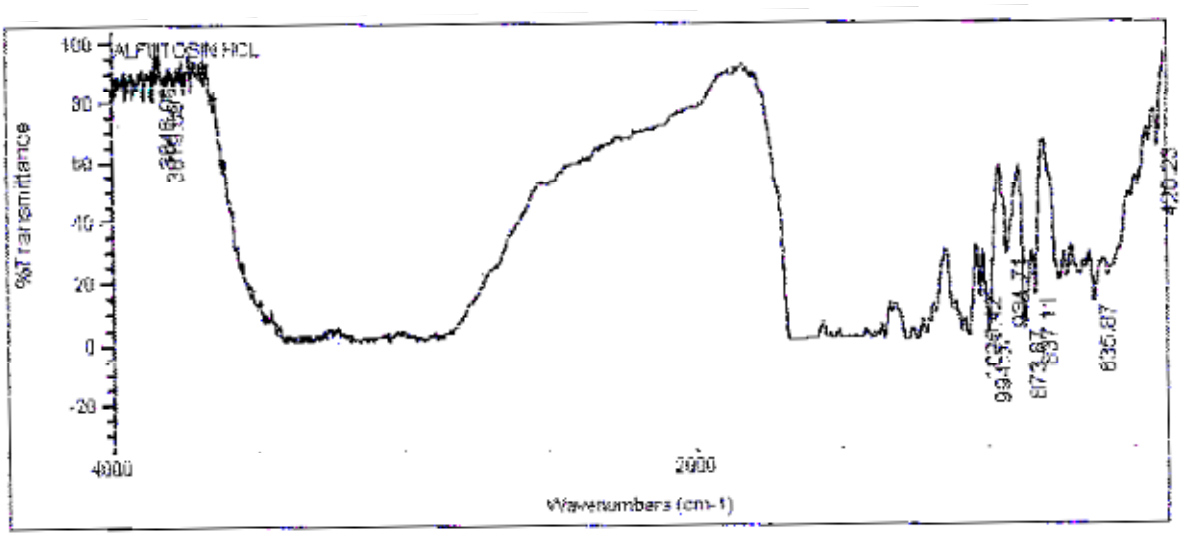
- Simultaneously, a newer RP-HPLC method was developed for both bulk drug and formulations. The proposed method gives reliable assay results with short analysis time (3.0 mins) using the mobile phase methanol: phosphate buffer-(p^H 3) at λ_{max} 245 nm. The content of drug present in the formulation, Alfoo was found to be 9.92 mg (99.20 %).

All the above methods do not suffer from any interference due to common excipients. Therefore it was found that the proposed methods could be successfully applied to estimate commercial pharmaceutical products containing Alfuzosin HC1. Thus, the above studies and findings will enable the quantification of the drug for future investigation in the field of analytical chemistry.

Figures

FIG -1

IR SPECTRUM OF ALFUZOSIN HC1



Mon Jan 28 10:08:38 2008
FIND PEAKS'

Spectrum: ALFUTOSIN HCL
Region: 4000.00 400.00
Absolute threshold: 103.305
Sensitivity: 27
Peak list:

Position: 1270.17 Intensity: -0.0017
Position: 3111.73 Intensity: -0.0766

Position: 1064.86 Intensity: 0.965
Position: 994.57 Intensity: 1.728
Position: 673.07 Intensity: 3.178
Position: 635.87 Intensity: 12.089
Position: 1025.42 Intensity: 14.073
Position: 837.11 Intensity: 14.513
Position: 934.71 Intensity: 27.335
Position: 420.23 Intensity: 68.170
Position: 3519.89 Intensity: 80.730
Position: 3648.89 Intensity: 86.829

FIG -2

UV- SPECTRUM OF ALFUZOSIN HC1 USING 0.1M NaOH

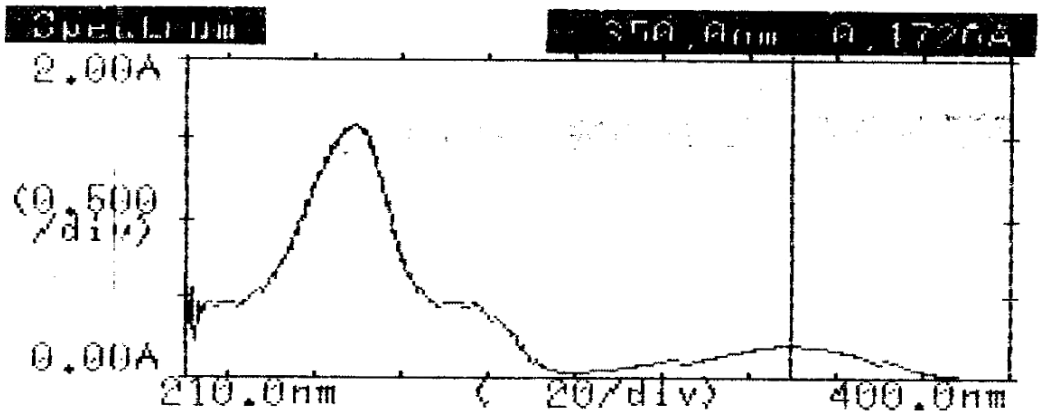
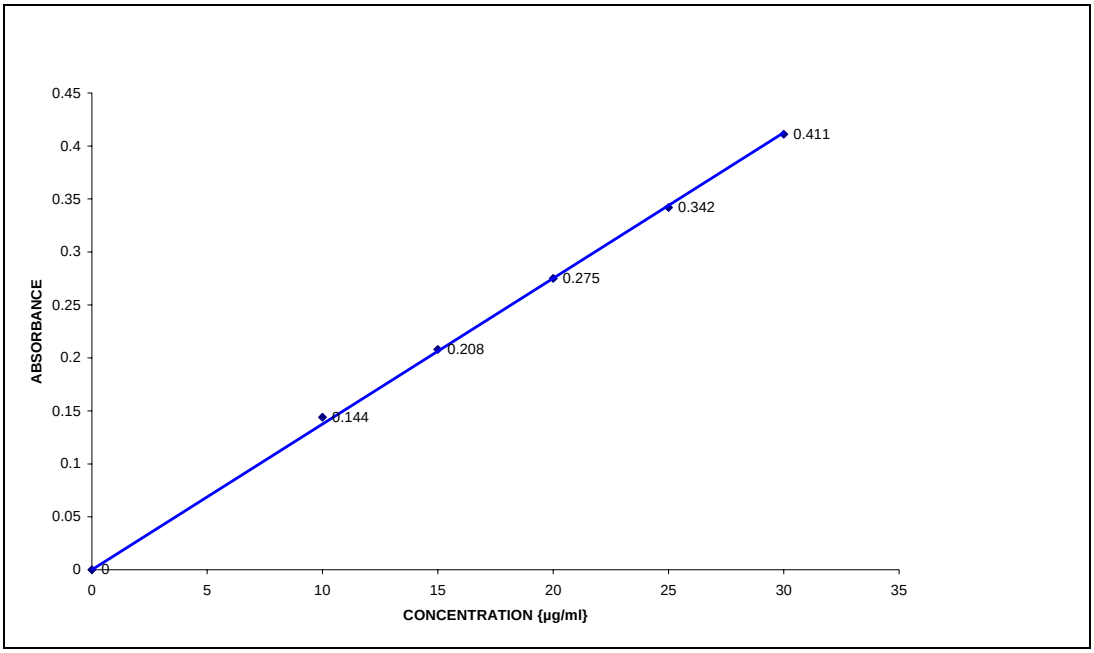


FIG -3

CALIBRATION CURVE FOR UV METHOD USING 0.1M NaOH



Relation between concentration and absorbance

FIG -4

VISIBLE ABSORPTION SPECTRUM OF ALFUZOSIN HC1 USING CHLOROFORM

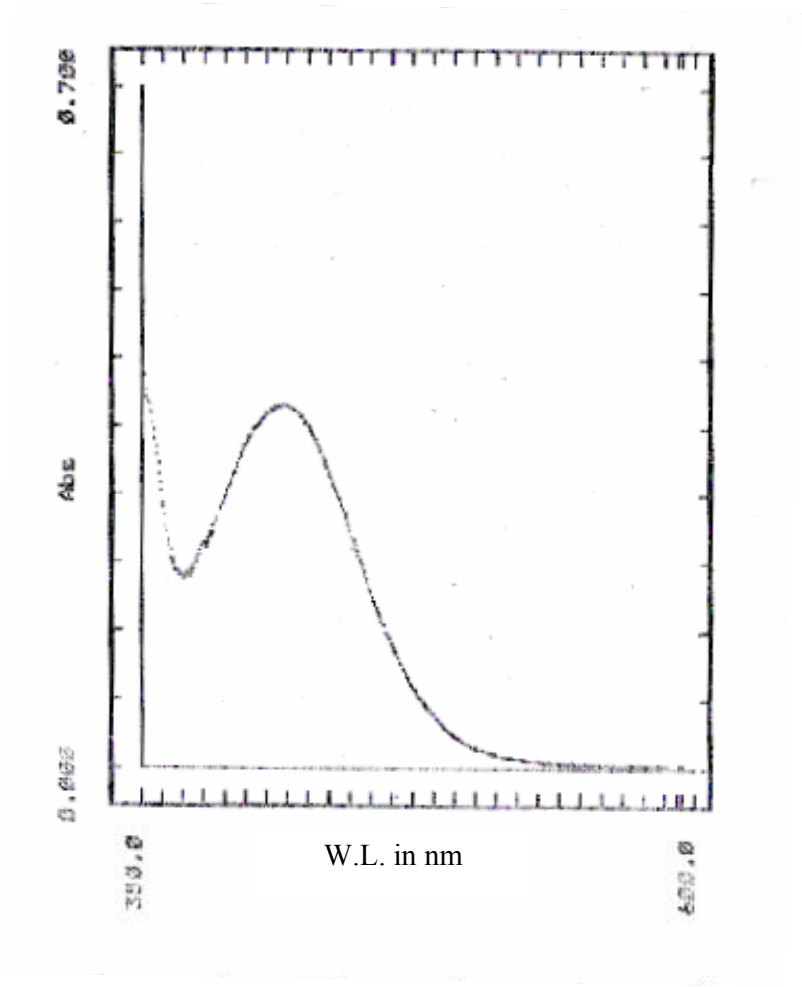
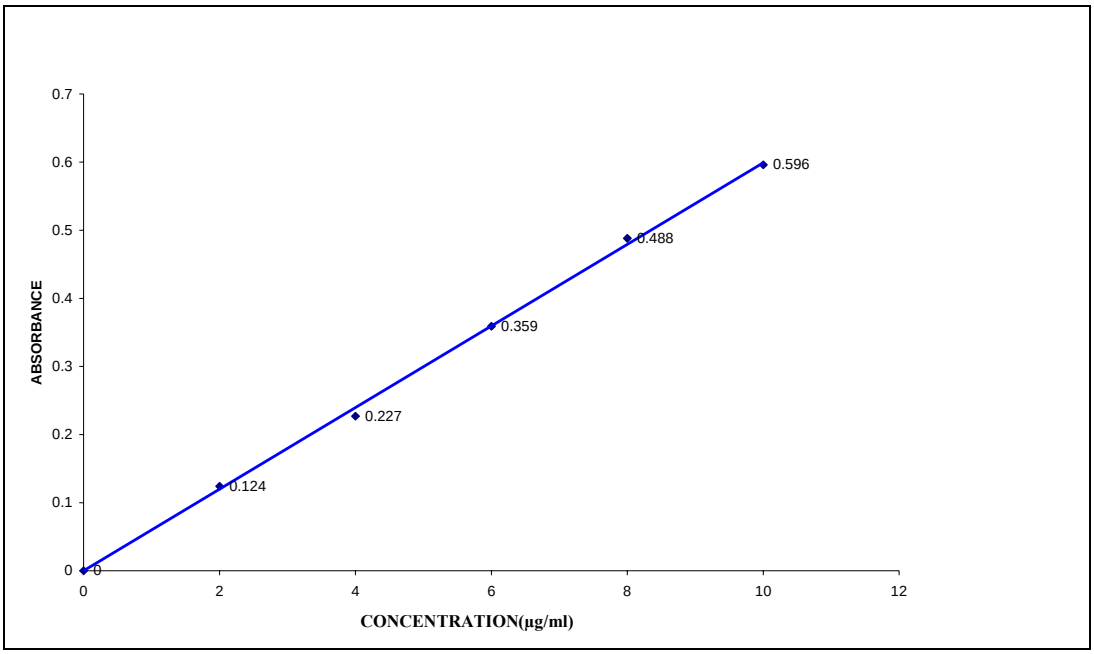


FIG -5

CALIBRATION CURVE OF VISIBLE METHOD OF ALFUZOSIN HC1 USING CHLOROFORM



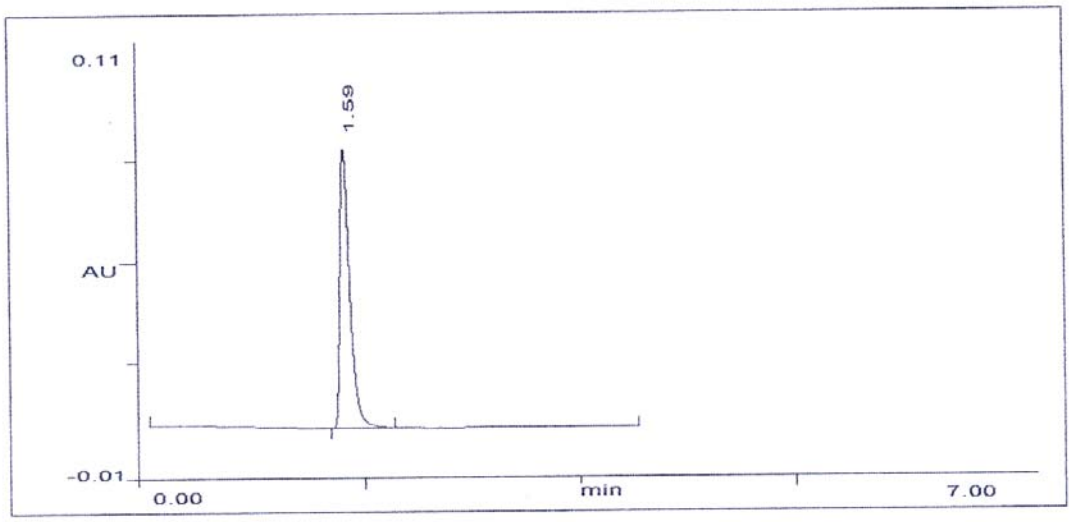
Relation between concentration and absorbance

FIG -6

OPTIMIZATION OF CHROMATOGRAM BY CHANGING MOBILE PHASE COMPOSITION METHANOL: BUFFER (p^H-3)

(80:20)

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	7.0



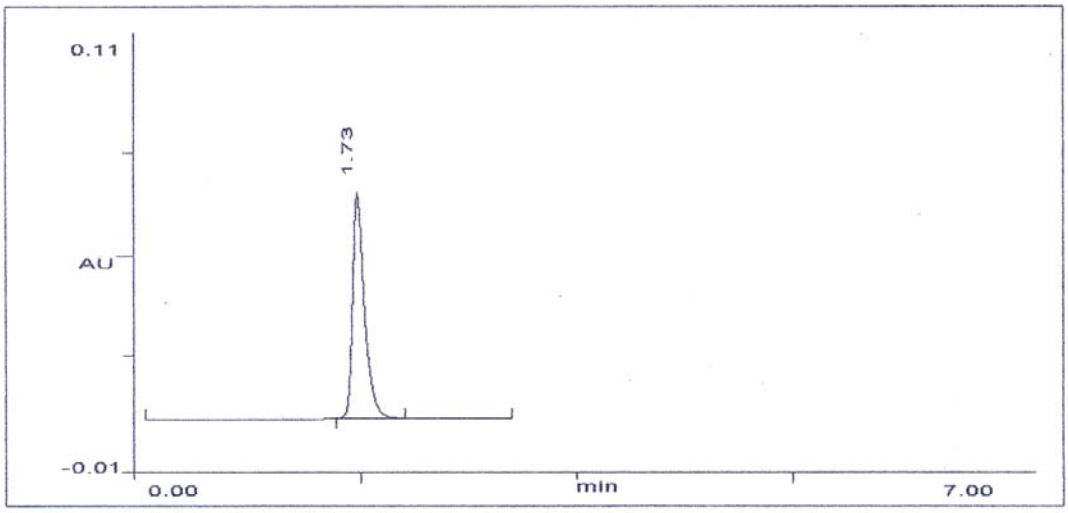
No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.59	18349	2472005	100.0000	100.0000	BB	0.092
		2e+04	2472005				

FIG -7

OPTIMIZATION OF CHROMATOGRAM BY CHANGING MOBILE PHASE COMPOSITION METHANOL: BUFFER (p^H-3)

(70:30)

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	7.0



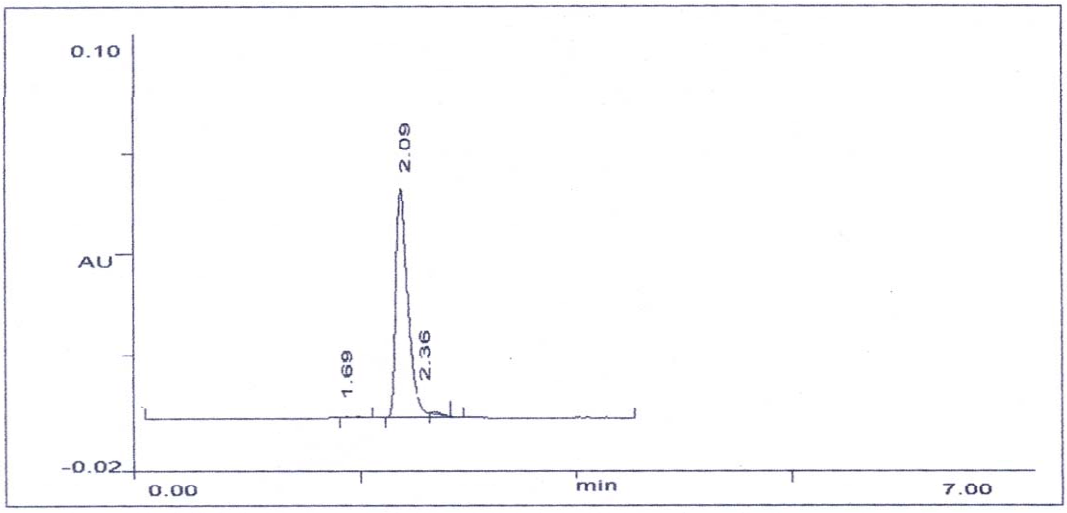
No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.73	14649	2382056	100.0000	100.0000	BB	0.111
		1e+04	2382056				

FIG -8

OPTIMIZATION OF CHROMATOGRAM BY CHANGING MOBILE PHASE COMPOSITION METHANOL: BUFFER (p^H-3)

(60:40)

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time S
4	30	5	4	7.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.69	166	20317	1.0841	0.8051	BB	0.084
2	2.09	15009	2486792	98.0212	98.5386	BB	0.113
3	2.36	137	16563	0.8947	0.6563	TTT	0.083
		2e+04	2523672				

FIG -9

OPTIMIZED CHROMATOGRAM IN MOBILE PHASE METHANOL: BUFFER (p^H-3) (50:50)

REPORT

Sample Name: **Data File: ...CHR~1\Def06.Dat**

Method File: ALF.MET

Detector: UV-VIS. **System: HPLC**

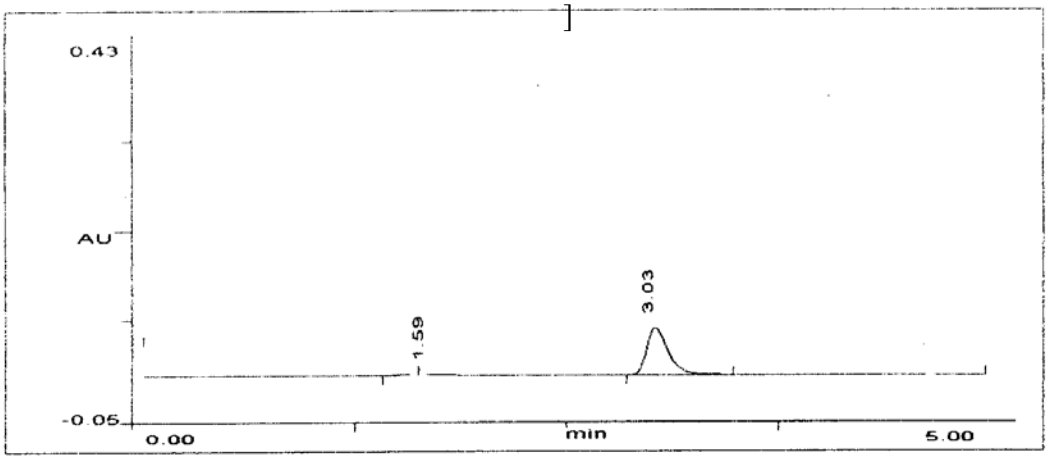
Date: 2 Jan 2008 **Time: 14:7:56**

Run: ch1: 2

Type of Analysis : Percent On Area and Height

Report printed on : 11/1/2008 at : 12:04:11

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	5.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.59	0	14068	0.0000	0.4793	BB	0.160
2	3.03	13843	2920835	100.0000	99.5207	BB	0.144
		1e+04	2934903				

No.	R.T.	Theo.Plt per unit length	Tailing Factor	Asym. Factor	Theo. Plates	Rel. Ret. time	Capa Fact
1	1.59	131.08	0.75	0.50	1966	0.00	0.03
2	3.03	206.00	1.45	1.71	3090	0.00	0.96

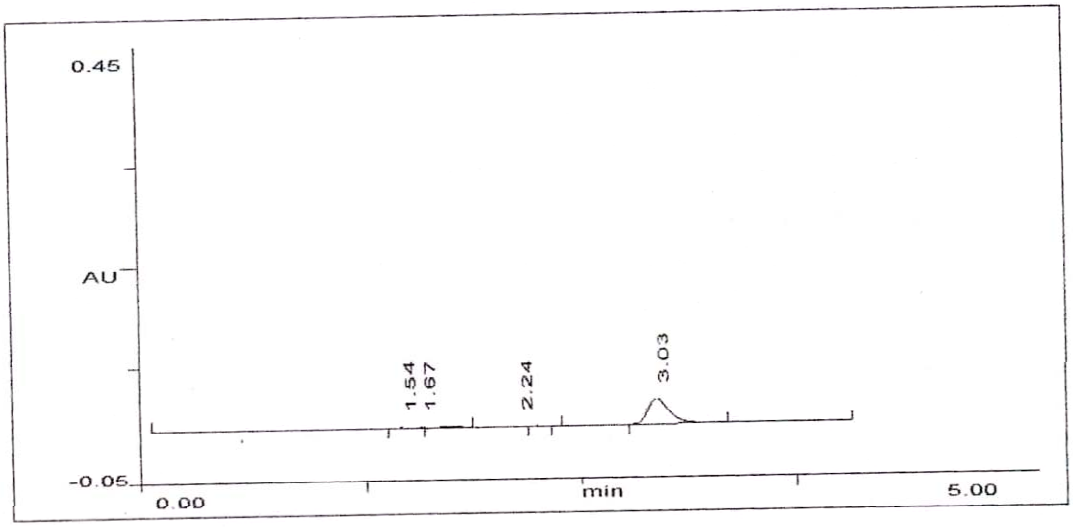
FIG -10

HPLC CHROMATOGRAM OF ALFUZOSIN HC1 IN MOBILE PHASE (1 µg/ml)

REPORT

Sample Name:
Data File: ...CHR~1\Def19.Dat
Method File: ALF.MET
Detector: UV-VIS.
System: HPLC
Date: 9 Jan 2008
Time: 12:24:36
Run: ch1: 4
Type of Analysis : Percent On Area and Height
Report printed on : 11/1/2008 at : 10:52:22

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	5.0



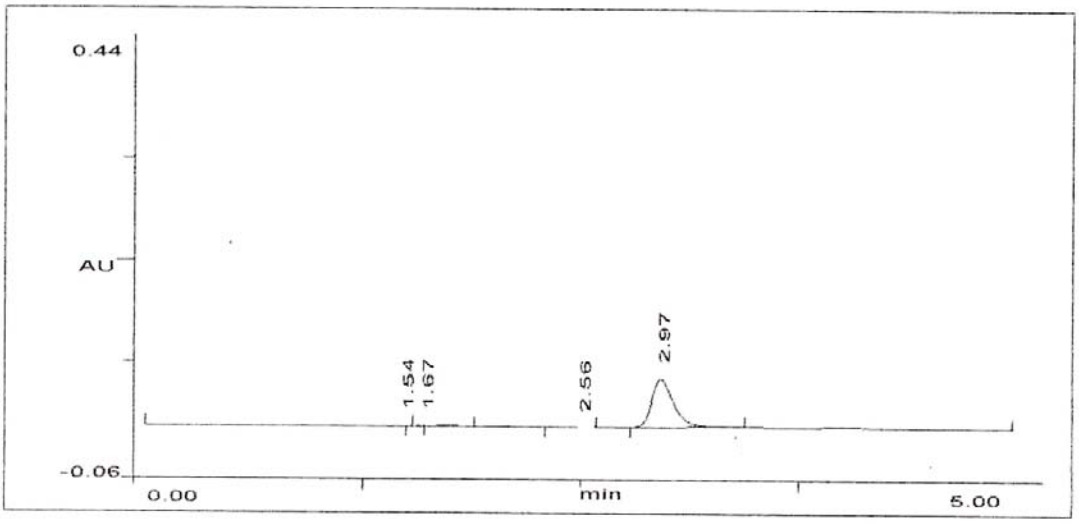
No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.54	133	25489	1.7966	1.6623	BV	0.131
2	1.67	216	43014	2.9177	2.8052	VB	0.136
3	2.24	64	3216	0.8645	0.2097	BP	0.034
4	3.03	6990	1461647	94.4212	95.3228	BB	0.143
		7e+03	1533366				

FIG -11
HPLC CHROMATOGRAM OF ALFUZOSIN HC1 IN MOBILE PHASE
(2 µg/ml)

REPORT

Sample Name:
Data File: ...CHR~1\Def16.Dat
Method File: ALF.MET
Detector: UV-VIS.
System: HPLC
Date: 9 Jan 2008
Time: 12:12:58
Run: ch1: 2
Type of Analysis : Percent On Area and Height
Report printed on : 11/1/2008 at : 10:44:22

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	5.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.54	0	5173	0.0000	0.1831	S	0.101
2	1.67	303	47185	2.2815	1.6702	BB	0.106
3	2.56	0	19601	0.0000	0.6938	BB	0.669
4	2.97	12978	2753153	97.7185	97.4529	BB	0.145
		1e+04	2825112				

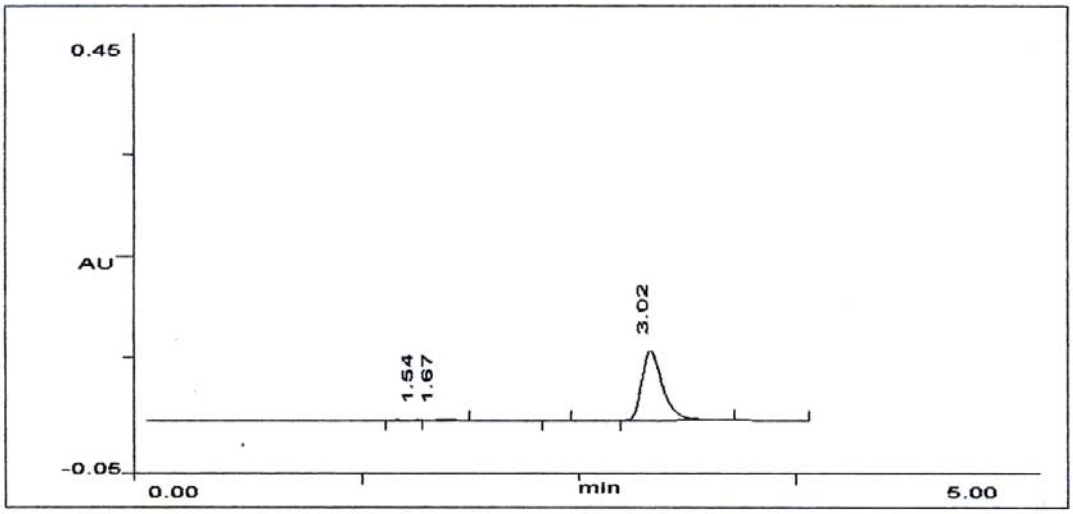
FIG -12

HPLC CHROMATOGRAM OF ALFUZOSIN HC1 IN MOBILE PHASE
(3 µg/ml)

REPORT

Sample Name: **Data File: ...CHR~1\Def20.Dat**
Method File: ALF.MET
Detector: UV-VIS. **System: HPLC**
Date: 9 Jan 2008 **Time: 12:24:36**
Run: ch1: 4
Type of Analysis : Percent On Area and Height
Report printed on : 11/1/2008 at : 10:52:22

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	5.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.54	137	26053	0.7116	0.6655	BV	0.130
2	1.67	234	42902	1.2154	1.0959	VB	0.125
3	3.02	18882	4384596	98.0730	98.2387	BB	0.139
		2e+04	4453551				

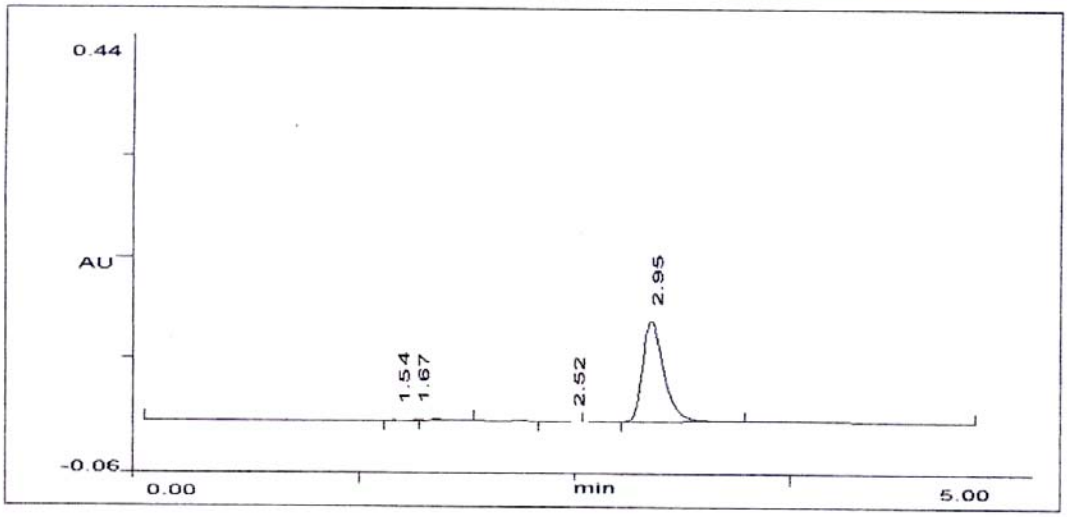
FIG -13

HPLC CHROMATOGRAM OF ALFUZOSIN HC1 IN MOBILE PHASE
(4 µg/ml)

REPORT

Sample Name:
Data File: ...CHR~1\Def17.Dat
Method File: ALF.MET
Detector: UV-VIS.
System: HPLC
Date: 9 Jan 2008
Time: 12:12:58
Run: ch1: 2
Type of Analysis : Percent On Area and Height
Report printed on : 11/1/2008 at : 10:44:22

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	5.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.54	172	28709	0.6082	0.4866	BV	0.114
2	1.67	539	95487	1.9060	1.6184	VB	0.121
3	2.52	0	19506	0.0000	0.3306	BB	0.476
4	2.95	27568	5756544	97.4858	97.5645	BB	0.143
		3e+04	5900246				

FIG -14

HPLC CHROMATOGRAM OF ALFUZOSIN HC1 IN MOBILE PHASE (5 µg/ml)

REPORT

Sample Name: Data File: ...CHR~1\Def25.Dat

Method File: ALF.MET

Detector: UV-VIS. System: HPLC

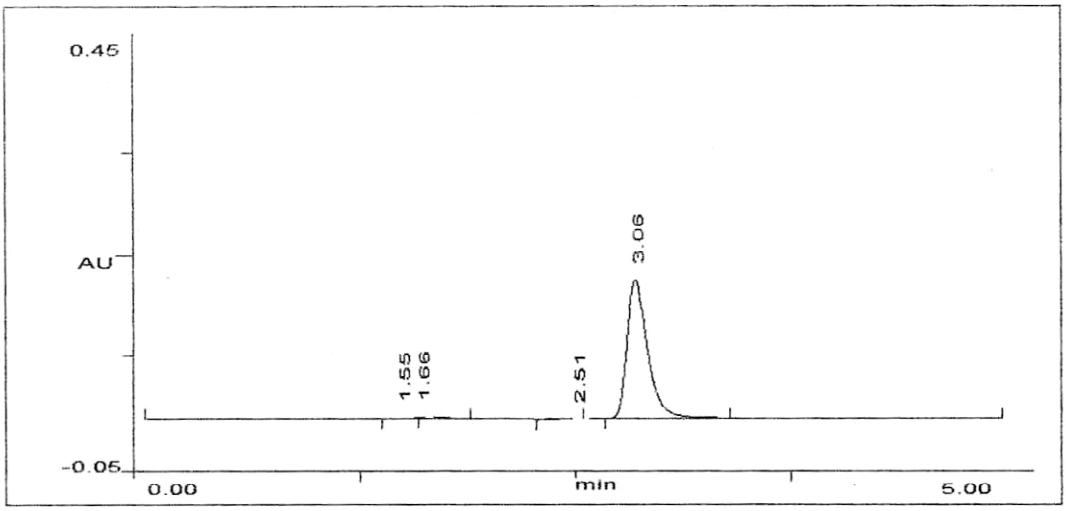
Date: 9 Jan 2008 Time: 14:19:40

Run: ch1: 4

Type of Analysis : Percent On Area and Height

Report printed on : 11/1/2008 at : 11:05:15

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	5.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.55	134	26069	0.3487	0.3392	BV	0.133
2	1.66	486	76038	1.2645	0.9893	VB	0.107
3	2.51	0	25838	0.0000	0.3362	BB	0.420
4	3.06	37813	7357781	98.3868	98.3353	BB	0.136
		4e+04	7485726				

FIG -15

CALIBRATION CURVE FOR RP-HPLC METHOD USING MOBILE PHASE

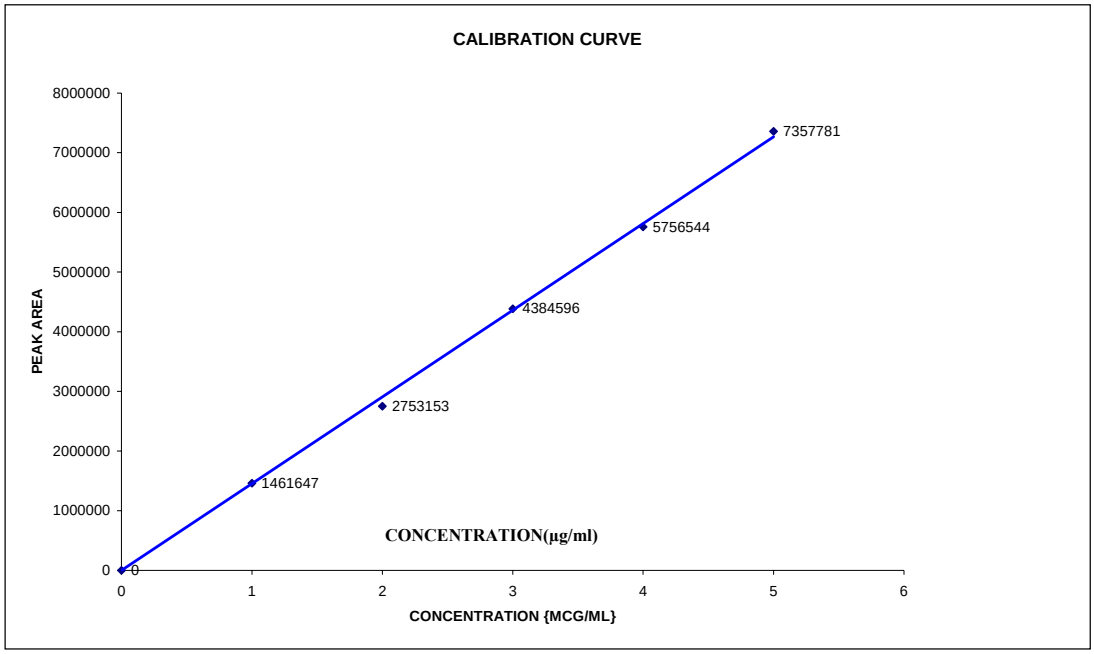
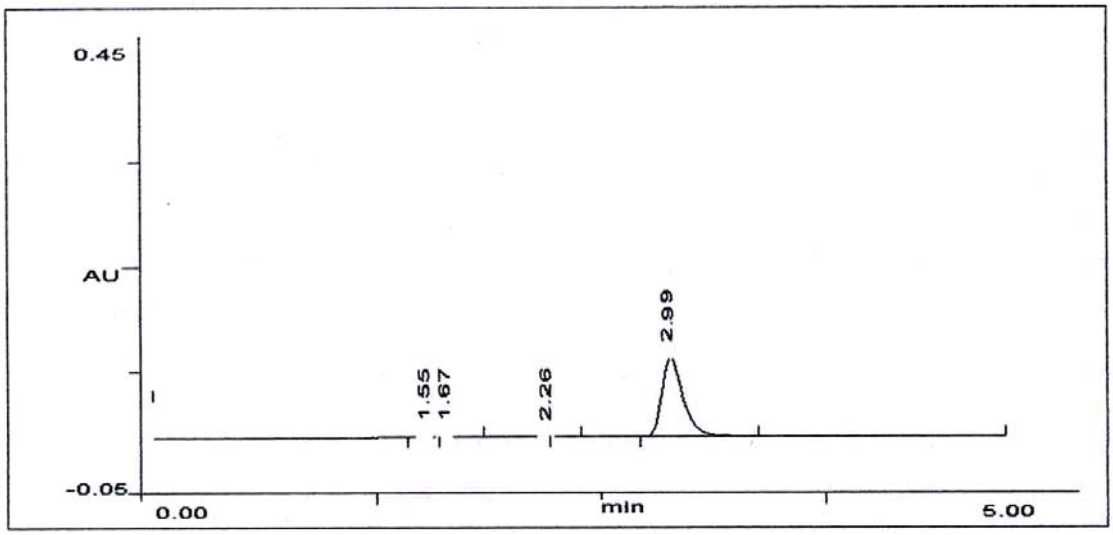


FIG -16
HPLC CHROMATOGRAM OF FORMULATION FOR REPEATABILITY I
ALFOO (3 µg/ml)

REPORT

Sample Name: **Data File: ...CHR~1\Def26.Dat**
Method File: ALF.MET
Detector: UV-VIS. **System: HPLC**
Date: 9 Jan 2008 **Time: 14:54:52**
Run: ch1: 11
Type of Analysis : Percent On Area and Height
Report printed on : 11/1/2008 at : 11:32:23

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	5.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.55	88	13964	0.4256	0.3366	BV	0.108
2	1.67	145	28824	0.7013	0.6949	VB	0.136
3	2.99	20442	4305123	98.8730	98.9684	BB	0.137
		2e+04	4347911				

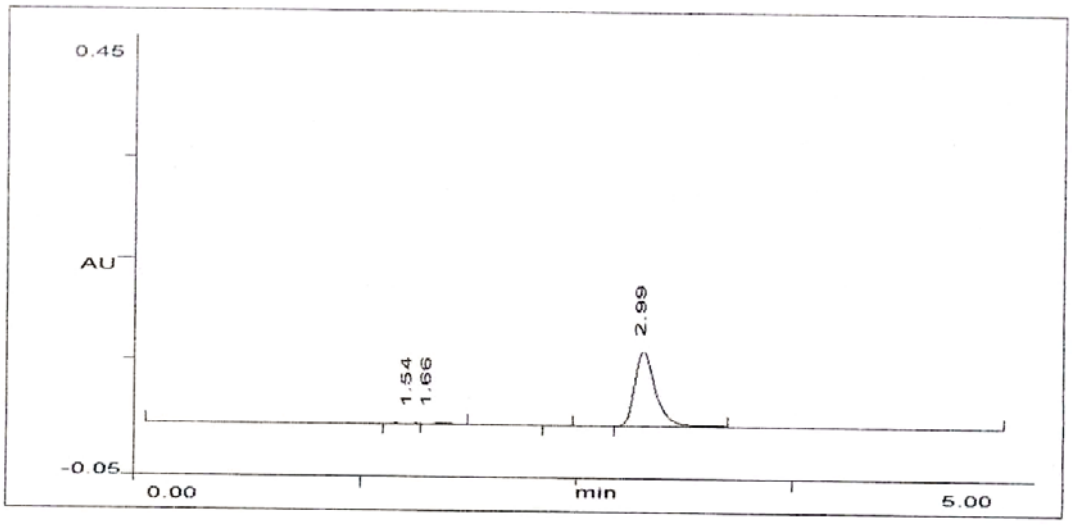
FIG -17

HPLC CHROMATOGRAM OF FORMULATION FOR REPEATABILITY II
ALFOO (3 µg/ml)

REPORT

Sample Name:
Data File: ...CHR~1\Def27.Dat
Method File: ALF.MET
Detector: UV-VIS.
System: HPLC
Date: 9 Jan 2008
Time: 14:31:48
Run: ch1: 6
Type of Analysis : Percent On Area and Height
Report printed on : 11/1/2008 at : 11:11:02

Pk.Width	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	5.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.54	130	25343	0.6286	0.6087	BV	0.133
2	1.66	257	45782	1.2427	1.0997	VB	0.122
3	2.99	20294	4392145	98.1287	98.2916	BB	0.138
		2e+04	4463270				

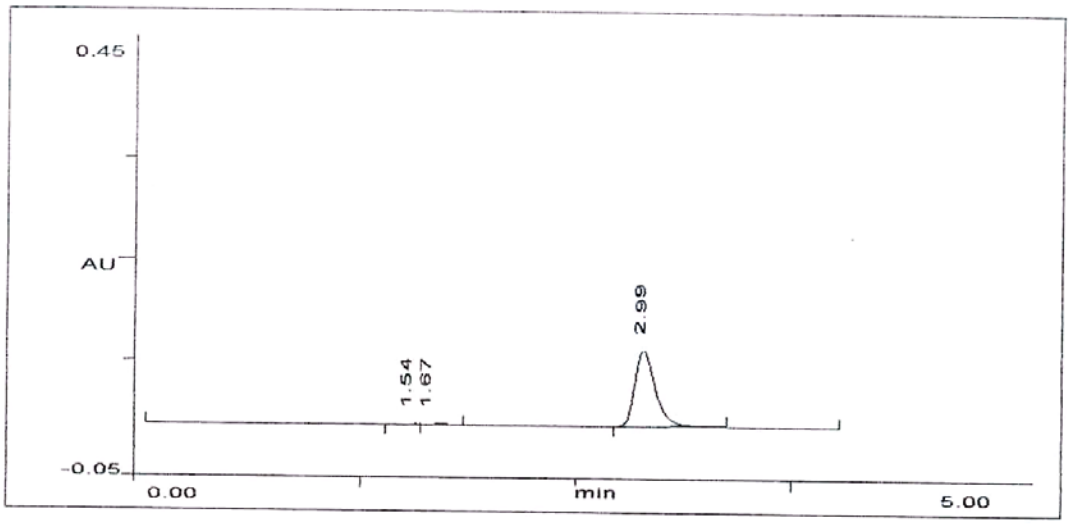
FIG -18

HPLC CHROMATOGRAM OF FORMULATION FOR REPEATABILITY III ALFOO (3 µg/ml)

REPORT

Sample Name:
Data File: ...CHR~1\Def28.Dat
Method File: ALF.MET
Detector: UV-VIS.
System: HPLC
Date: 9 Jan 2008
Time: 14:36:12
Run: ch1: 7
Type of Analysis : Percent On Area and Height
Report printed on : 11/1/2008 at : 11:14:18

Pk.Width	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	5.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.54	100	18110	0.4763	0.4273	BV	0.124
2	1.67	134	27357	0.6382	0.6455	VB	0.139
3	2.99	20761	4392663	98.8855	98.9272	BB	0.138
		2e+04	4438130				

FIG -19

HPLC CHROMATOGRAM OF FORMULATION FOR REPEATIBALITY IV
ALFOO (3 µg/ml)

REPORT

Sample Name: Data File: ...CHR~1\Def29.Dat

Method File: ALF.MET

Detector: UV-VIS. System: HPLC

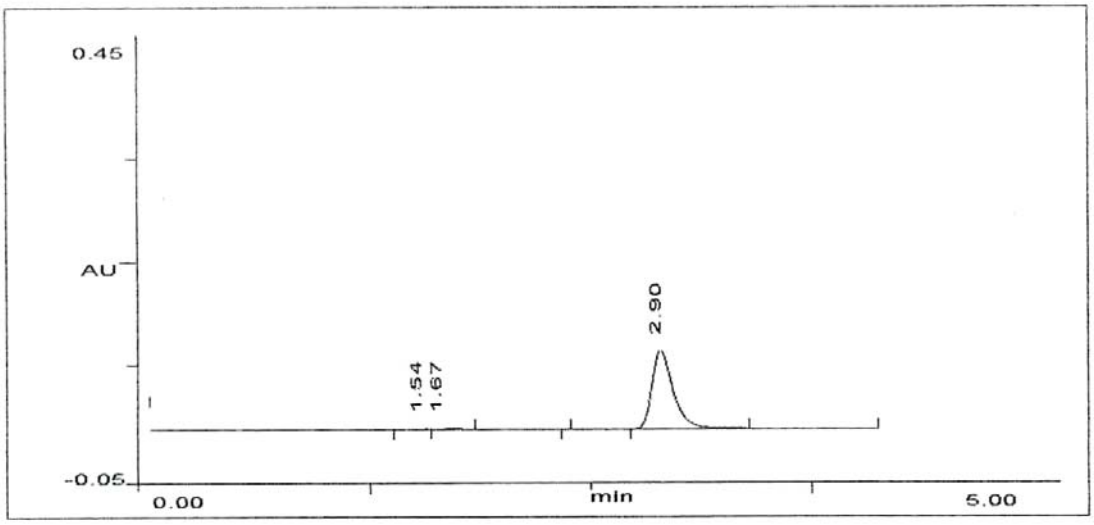
Date: 9 Jan 2008 Time: 14:40:32

Run: ch1: 8

Type of Analysis : Percent On Area and Height

Report printed on : 11/1/2008 at : 11:17:28

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	5.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.54	115	21457	0.5475	0.5025	BV	0.127
2	1.67	165	32055	0.7856	0.7506	VB	0.133
3	2.90	20724	4216842	98.6669	98.7469	BB	0.139
		2e+04	4270354				

FIG -20

HPLC CHROMATOGRAM OF FORMULATION FOR REPEATIBALITY V ALFOO (3 µg/ml)

REPORT

Sample Name: Data File: ...CHR~1\Def30.Dat

Method File: ALF.MET

Detector: UV-VIS. System: HPLC

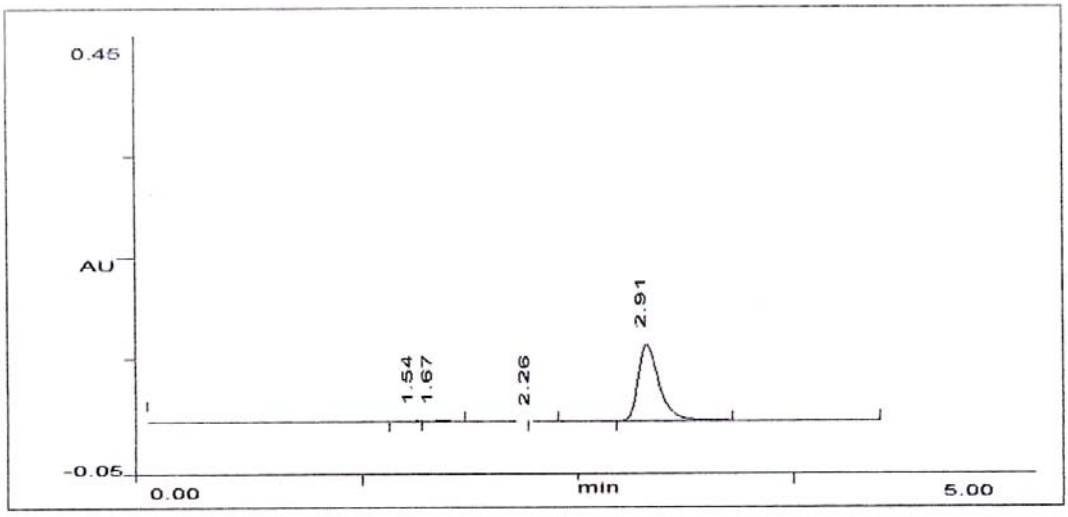
Date: 9 Jan 2008 Time: 14:45:4

Run: ch1: 9

Type of Analysis : Percent On Area and Height

Report printed on : 11/1/2008 at : 11:28:01

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	5.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.54	96	17284	0.4594	0.4063	BV	0.123
2	1.67	133	26941	0.6364	0.6333	VB	0.138
3	2.91	20669	4209694	98.9042	98.9604	BB	0.139
		2e+04	4253919				

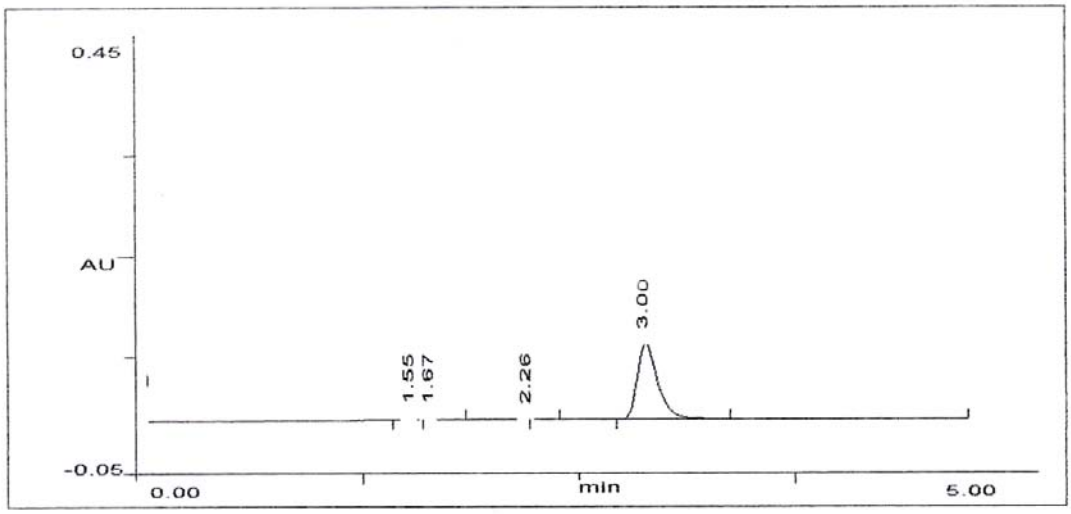
FIG -21

HPLC CHROMATOGRAM OF FORMULATION FOR REPEATABILITY VI
ALFOO (3 µg/ml)

REPORT

Sample Name: Data File: ...CHR~1\Def32.Dat
Method File: ALF.MET
Detector: UV-VIS. System: HPLC
Date: 9 Jan 2008 Time: 14:54:52
Run: ch1: 11
Type of Analysis : Percent On Area and Height
Report printed on : 11/1/2008 at : 11:32:23

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	5.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.55	88	13964	0.4256	0.3366	BV	0.108
2	1.67	145	28824	0.7013	0.6949	VB	0.136
3	3.00	20442	4305123	98.8730	98.9684	BB	0.137
		2e+04	4347911				

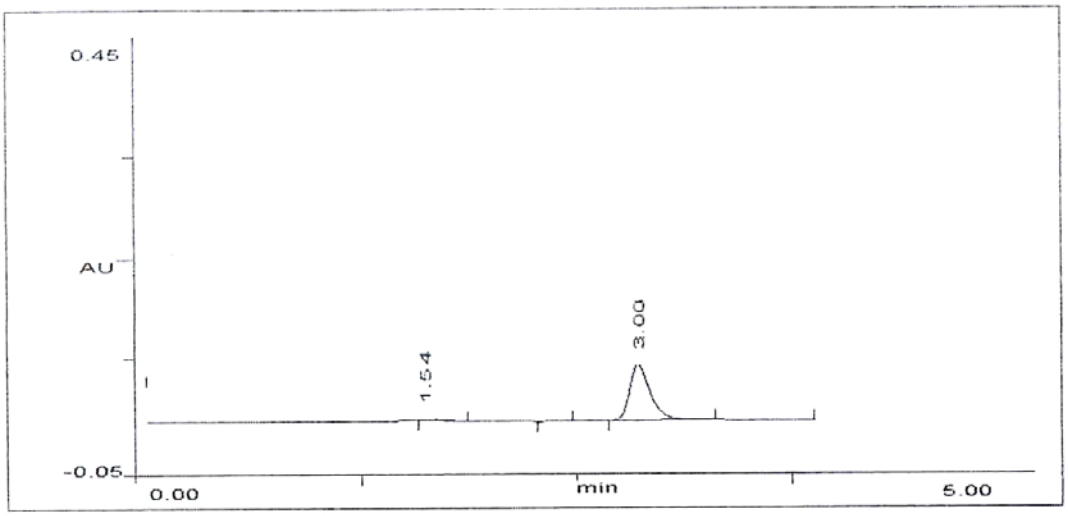
FIG -22

HPLC CHROMATOGRAM FOR THE RECOVERY OF FORMULATION (ALFOO) (2 µg/ml)

REPORT

Sample Name:
Data File: ...CHR~1\Def33.Dat
Method File: ALF.MET
Detector: UV-VIS.
System: HPLC
Date: 9 Jan 2008
Time: 14:59:28
Run: ch1: 12
Type of Analysis : Percent On Area and Height
Report printed on : 11/1/2008 at : 11:34:57

Pk.Width	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	5.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.54	443	54844	2.8729	1.8007	BB	0.085
2	3.00	14977	2790802	97.1271	98.1993	BB	0.136
		2e+04	2845646				

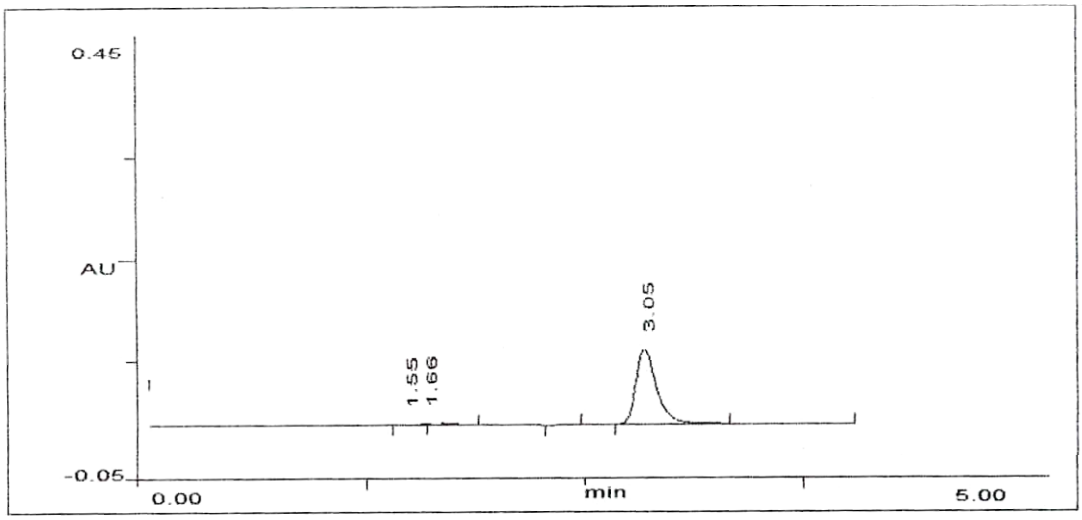
FIG -23

HPLC CHROMATOGRAM FOR THE RECOVERY OF FORMULATION (ALFOO)
(2. 5 µg/ml)

REPORT

Sample Name:
Data File: ...CHR~1\Def34.Dat
Method File: ALF.MET
Detector: UV-VIS.
System: HPLC
Date: 9 Jan 2008
Time: 15:3:48
Run: ch1: 13
Type of Analysis : Percent On Area and Height
Report printed on : 11/1/2008 at : 11:37:45

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	5.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.55	95	17944	0.4560	0.4395	BV	0.129
2	1.66	574	78286	2.7552	1.9176	VB	0.093
3	3.05	20164	3486346	96.7887	97.6429	BB	0.135
		2e+04	3582576				

FIG -24

HPLC CHROMATOGRAM FOR THE RECOVERY OF FORMULATION (ALFOO) (3 µg/ml)

REPORT

Sample Name: Data File: ...CHR~1\Def35.Dat

Method File: ALF.MET

Detector: UV-VIS. System: HPLC

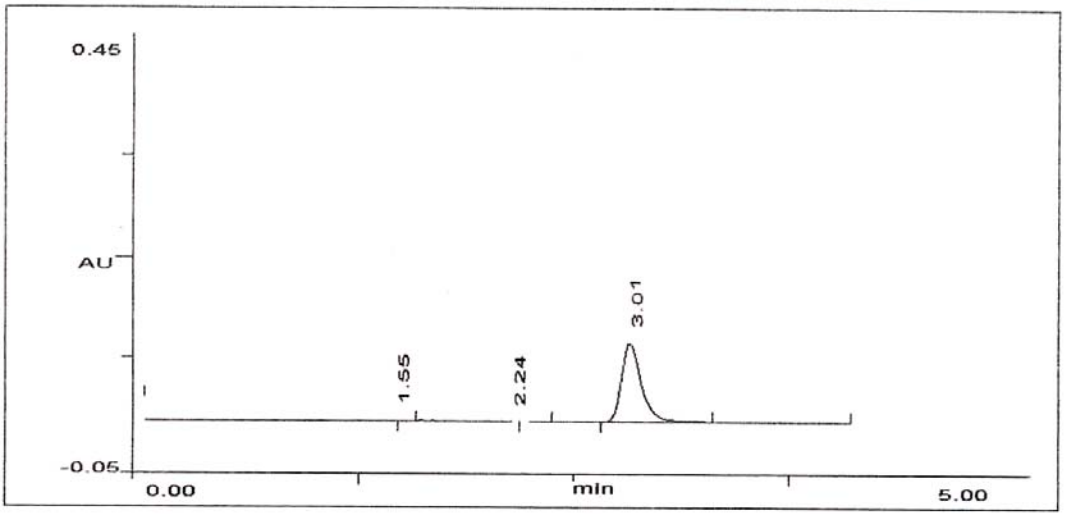
Date: 9 Jan 2008 Time: 15:8:26

Run: ch1: 14

Type of Analysis : Percent On Area and Height

Report printed on : 11/1/2008 at : 11:41:49

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	5.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.55	0	2395	0.0000	0.0583	BB	0.071
2	2.24	0	3514	0.0000	0.0856	BB	0.041
3	3.01	21183	4398731	100.0000	99.8560	BB	0.132
		2e+04	4404640				

FIG -25

HPLC CHROMATOGRAM FOR THE RECOVERY OF FORMULATION (ALFOO) (3.5 µg/ml)

REPORT

Sample Name: **Data File: ...CHR~1\Def36.Dat**

Method File: ALF.MET

Detector: UV-VIS. **System: HPLC**

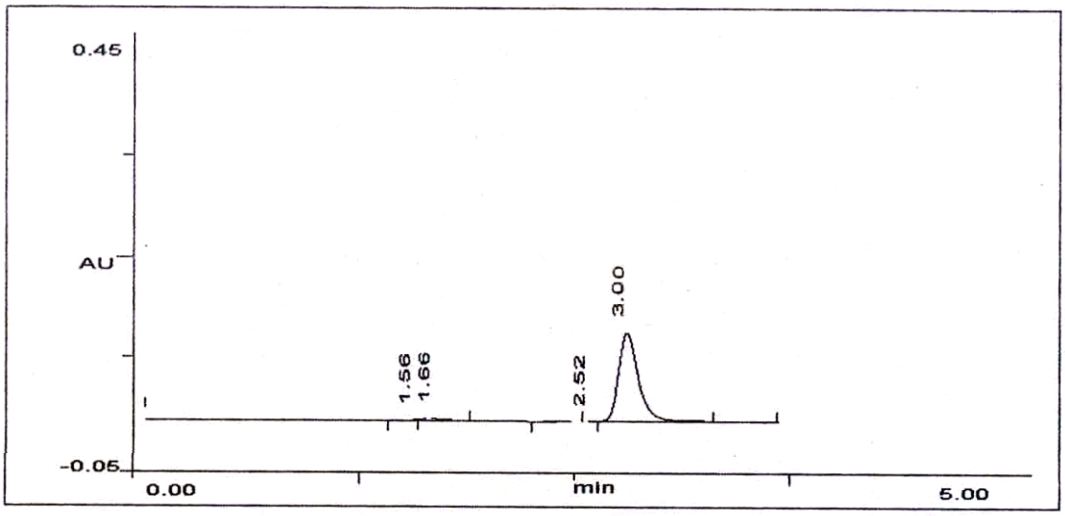
Date: 9 Jan 2008 **Time: 15:12:22**

Run: ch1: 15

Type of Analysis : Percent On Area and Height

Report printed on : 11/1/2008 at : 11:43:32

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	5.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.56	93	16105	0.3740	0.3341	BV	0.118
2	1.66	670	93152	2.6943	1.9327	VB	0.095
3	2.52	0	43985	0.0000	0.9126	BB	0.698
4	3.00	24104	4866578	96.9317	96.8206	BB	0.132
		2e+04	5019820				

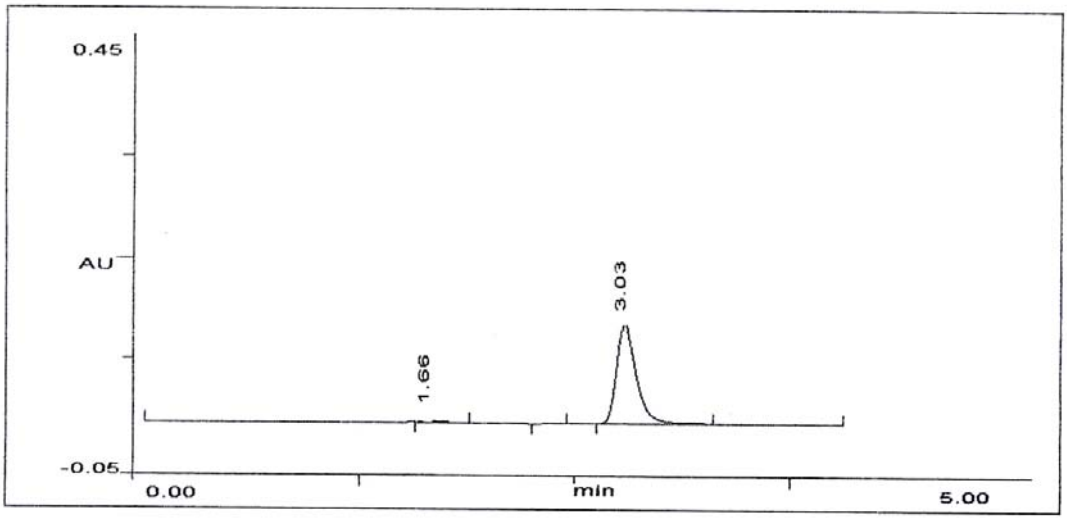
FIG -26

HPLC CHROMATOGRAM FOR THE RECOVERY OF FORMULATION (ALFOO) (4 µg/ml)

REPORT

Sample Name:
Data File: ...CHR~1\Def37.Dat
Method File: ALF.MET
Detector: UV-VIS.
System: HPLC
Date: 9 Jan 2008
Time: 15:16:38
Run: ch1: 16
Type of Analysis : Percent On Area and Height
Report printed on : 11/1/2008 at : 11:47:24

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	5.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.66	513	62889	1.8718	1.2034	BB	0.084
2	3.03	26894	5763065	98.1282	98.7966	BB	0.131
		3e+04	5825954				

FIG-27

HPLC CHROMATOGRAM FOR THE RECOVERY OF FORMULATION (ALFOO) (4.5 µg/ml)

REPORT

Sample Name: **Data File: ...CHR~1\Def38.Dat**

Method File: ALF.MET

Detector: UV-VIS. **System: HPLC**

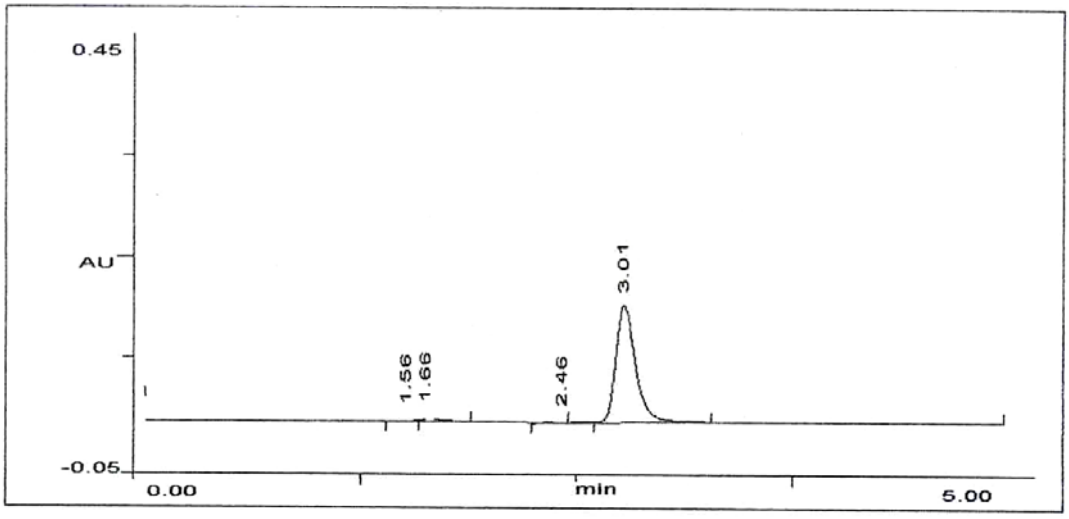
Date: 9 Jan 2008 **Time: 15:21:50**

Run: ch1: 17

Type of Analysis : Percent On Area and Height

Report printed on : 11/1/2008 at : 11:52:05

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	5.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.56	100	18622	0.3034	0.2900	BV	0.127
2	1.66	751	100984	2.2786	1.5727	VB	0.092
3	2.46	350	148970	1.0619	2.3200	S	0.291
4	3.01	31758	6382542	96.3561	95.8173	BB	0.132
		3e+04	6651118				

Tables

TABLE – 1

RESULTS OF SOLUBILITY OF ALFUZOSIN HC1 IN POLAR AND NONPOLAR SOLVENTS

S.No.	SOLVENTS	STATUS
1.	Water	Very soluble
2.	0.1N HCl	Very soluble
3.	0.1N NaOH	Very soluble
4.	Methanol	Very soluble
5.	Ethanol	Freely soluble
6.	Acetonitrile	Freely soluble
7.	Dimethyl formamide	Freely soluble
8.	Acetone	Very soluble
9.	Chloroform	Soluble
10.	Ethyl acetate	Insoluble
11.	Dichloroethane	Insoluble
12	Diethyl ether	Insoluble

TABLE - 2

OPTICAL CHARACTERISTICS OF ALFUZOSIN HC1 BY UV METHOD

Parameters	Values
λ_{max}(nm)	350
Beers law limit (μg/ml)	10-30
Sandell's sensitivity (μg/cm^2/0.001 A.U)	0.074850299
Molar absorptivity (L mol^{-1} cm^{-1})	5545.056
Correlation coefficient (r)	0.999914
Regression equation (y = mx + c)	Y = 0.01336 X + 0.0088
Slope(m)	0.01336
Intercept(c)	0.0088
LOD (μg/ml)	2.57
LOQ (μg/ml)	7.793
Standard error of mean of Regression line	0.001591645

TABLE – 3

QUANTIFICATION OF FORMULATION –ALFOO

BY UV METHOD

S.No	Labeled amount (mg/tab)	Amount found(mg)	Percentage obtained	Average	S.D.	%RSD	S.E.
1.	10.00	9.80	98.00	98.10	0.1629	0.1660	0.004
2.	10.00	9.83	98.30				
3.	10.00	9.80	98.00				
4.	10.00	9.80	98.00				
5.	10.00	9.80	98.00				
6.	10.00	9.83	98.30				

TABLE – 4

QUANTIFICATION OF FORMULATION –ALFUSIN

BY UV METHOD

S.No	Labeled amount (mg/tab)	Amount found(mg)	Percentage obtained	Average	S.D.	%RSD	S.E.
1.	10.00	9.80	98.00	99.68	1.315	1.31	0.036
2.	10.00	10.00	100.00				
3.	10.00	10.10	101.00				
4.	10.00	9.80	98.00				
5.	10.00	9.96	99.60				
6.	10.00	10.15	101.50				

TABLE - 5

QUANTIFICATION OF FORMULATION –XELFLO

BY UV METHOD

S.No	Labeled amount (mg/tab)	Amount found(mg)	Percentage obtained	Average	S.D.	%RSD	S.E.
1.	10.00	10.06	100.60				
2.	10.00	9.91	99.10				
3.	10.00	9.91	99.10	100.00	1.188	1.187	0.033
4.	10.00	9.91	99.10				
5.	10.00	10.13	101.30				
6.	10.00	10.10	101.00				

TABLE – 6

RECOVERY STUDIES FOR FORMULATION - ALFOO

BY UV METHOD

S.No	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Estimated (µg/ml)	Amount Recovered (µg/ml)	% Recovery	S.D.	%RSD	S.E.
1.	9.95	5	14.76	4.84	102.00			
2.	9.90	6	15.46	5.58	99.50			
3.	9.84	7	16.90	7.12	100.80	1.668	1.663	0.046
4.	9.84	8	17.73	7.89	99.01			
5.	9.83	9	18.54	8.75	100.24			
6.	9.80	10	19.62	9.82	98.80			
				Mean	100.05			

TABLE – 7

RECOVERY STUDIES FOR FORMULATION – ALFUSIN

BY UV METHOD

S.No	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Estimated (µg/ml)	Amount Recovered (µg/ml)	% Recovery	S.D.	%RSD	S.E.
1.	9.94	5	14.65	4.72	99.50			
2.	9.94	6	16.03	6.01	100.60			
3.	9.94	7	16.52	6.63	101.70	0.957	0.951	0.0026
4.	9.98	8	17.77	7.75	100.60			
5.	9.94	9	18.58	8.62	102.08			
6.	10.02	10	19.98	9.96	100.43			
				Mean	100.81			

TABLE – 8

RECOVERY STUDIES FOR FORMULATION – XELFLO

BY UV METHOD

S.No	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Estimated (µg/ml)	Amount Recovered (µg/ml)	% Recovery	S.D.	%RSD	S.E.
1.	9.82	5	14.65	4.87	98.10			
2.	9.80	6	15.47	5.68	101.10			
3.	9.85	7	16.36	6.62	98.00	1.454	1.462	0.0404
4.	9.81	8	17.49	7.68	100.80			
5.	9.83	9	18.51	8.70	98.44			
6.	9.83	10	19.61	9.78	100.50			
				Mean	99.49			

TABLE – 9

INTER DAY AND INTRADAY ANALYSIS OF FORMULATION ALFOO BY UV METHOD

S.No	**INTERDAY** (Amount found in mg)	**INTRA DAY** (Amount found in mg)
1.	9.80	9.83
2.	9.80	9.85
3.	9.85	9.86
4.	9.85	9.80
5.	9.83	9.80
6.	9.88	9.93
S.D	0.03355	0.0485
%RSD	0.3410	0.4925

TABLE – 10

INTERDAY AND INTRADAY ANALYSIS OF FORMULATION – ALFUSIN BY UV METHOD

S.No	**INTERDAY** (Amount found in mg)	**INTRA DAY** (Amount found in mg)
1.	9.80	9.81
2.	9.81	9.80
3.	9.80	9.80
4.	9.84	9.87
5.	9.80	9.80
6.	9.80	9.88
S.D	0.0178	0.0392
%RSD	0.1824	0.3991

TABLE – 11

INTER DAY AND INTRA DAY ANALYSIS OF FORMULATION – XELFLO BY UV METHOD

S.No	**INTERDAY** (Amount found in mg)	**INTRA DAY** (Amount found in mg)
1.	9.81	9.86
2.	9.82	9.80
3.	9.81	9.82
4.	9.83	9.83
5.	9.87	9.80
6.	9.80	9.84
S.D	0.0250	0.0240
%RSD	0.2548	0.2448

TABLE - 12

OPTICAL CHARACTERISTICS OF ALFUZOSIN HC1 BY COLORIMETRIC METHOD

Parameters	Values
λ_{max}(nm)	416
Beers law limit (µg/ml)	2-10
Sandell's sensitivity (µg/cm^2/0.001 A.U)	0.016706348
Molar absorptivity (L mol^{-1} cm^{-1})	23319.1881
Correlation coefficient (r)	0.999588
Regression equation (y = mx+c)	Y=0.059864286X + 0.000206349
Slope(m)	0.059864286
Intercept(c)	0.000206349
LOD (µg/ml)	0.180706281
LOQ (µg/ml)	0.547594
Standard error of mean of Regression line	0.000518438

TABLE – 13

QUANTIFICATION OF FORMULATION –ALFOO

BY COLORIMETRIC METHOD

S.No	Labeled amount (mg/tab)	Amount found(mg)	Percentage obtained	Average	S.D.	%RSD	S.E.
1.	10.00	9.86	98.60	99.28	1.5819	1.5814	0.0439
2.	10.00	10.01	100.10				
3.	10.00	9.98	99.80				
4.	10.00	10.02	100.20				
5.	10.00	9.90	99.00				
6.	10.00	9.80	98.00				

TABLE – 14

QUANTIFICATION OF FORMULATION –ALFUSIN

BY COLORIMETRIC METHOD

S.No	Labeled amount (mg/tab)	Amount found(mg)	Percentage obtained	Average	S.D.	%RSD	S.E.
1.	10.00	9.95	99.50	100.00	0.9230	0.9230	0.0256
2.	10.00	10.10	101.00				
3.	10.00	9.98	99.80				
4.	10.00	10.10	101.00				
5.	10.00	10.01	100.10				
6.	10.00	9.86	98.60				

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TABLE – 15

QUANTIFICATION OF FORMULATION –XELFLO

BY COLORIMETRIC METHOD

S.No	Labeled amount (mg/tab)	Amount found(mg)	Percentage obtained	Average	S.D.	%RSD	S.E.
1.	10.00	9.82	98.20	98.40	0.9042	0.9178	0.0251
2.	10.00	9.80	98.00				
3.	10.00	9.80	98.00				
4.	10.00	9.80	98.00				
5.	10.00	9.80	98.00				
6.	10.00	10.03	100.30				

TABLE – 16

RECOVERY STUDIES FOR FORMULATION ALFOO

BY COLORIMETRIC METHOD

S.No	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Estimated (µg/ml)	Amount Recovered (µg/ml)	% Recovery	S.D.	%RSD	S.E.
1.	2.95	1	3.819	0.871	98.00			
2.	2.95	2	4.729	1.778	100.20			
3.	2.95	3	5.882	2.932	98.80	1.7713	1.7835	0.0492
4.	2.95	4	6.793	3.844	98.80			
5.	2.95	5	7.853	4.916	98.40			
6.	2.95	6	8.699	5.759	100.80			
				Mean	99.16			

TABLE – 17

RECOVERY STUDIES FOR FORMULATION – ALFUSIN

BY COLORIMETRIC METHOD

S.No	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Estimated (µg/ml)	Amount Recovered (µg/ml)	% Recovery	S.D.	%RSD	S.E.
1.	2.90	1	3.885	0.935	98.30			
2.	2.90	2	4.710	1.768	102.50			
3.	2.90	3	5.834	2.8845	99.66	1.6933	1.6967	0.0470
4.	2.90	4	6.806	3.85635	98.03			
5.	2.90	5	7.875	4.924	99.37			
6.	2.90	6	8.785	5.836	101.09			
				Mean	99.82			

TABLE -18

RECOVERY STUDIES FOR FORMULATION – XELFLO

BY COLORIMETRIC METHOD

S.No	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Estimated (µg/ml)	Amount Recovered (µg/ml)	% Recovery	S.D.	%RSD	S.E.
1.	2.95	1	3.813	0.862	102.00			
2.	2.95	2	4.492	1.542	98.00			
3.	2.95	3	5.661	2.726	98.00	1.90	1.917	0.0528
4.	2.95	4	6.500	3.522	100.00			
5.	2.95	5	7.815	4.865	99.80			
6.	2.95	6	8.591	5.663	99.20			
				Mean	99.50			

TABLE – 19

INTERDAY AND INTRADAY ANALYSIS OF FORMULATION – ALFOO BY COLORIMETRIC METHOD

S.No	**INTERDAY** (Amount found in mg)	**INTRA DAY** (Amount found in mg)
1.	10.01	9.94
2.	10.08	10.06
3.	9.89	10.03
4.	9.99	10.03
5.	9.89	9.96
6.	9.94	9.90
S.D	0.0909	0.0625
%RSD	0.9136	0.6258

TABLE – 20

INTERDAY AND INTRADAY ANALYSIS OF FORMULATION –ALFUSIN BY COLORIMETRIC METHOD

S.No	**INTERDAY** (Amount found in mg)	**INTRA DAY** (Amount found in mg)
1.	9.94	10.06
2.	9.96	10.00
3.	9.86	10.07
4.	9.90	10.06
5.	10.50	10.13
6.	10.03	10.13
S.D	0.1075	0.0492
%RSD	1.078	0.4892

TABLE – 21

INTERDAY AND INTRADAY ANALYSIS OF FORMULATION – XELFLO BY COLORIMETRIC METHOD

S.No	**INTERDAY** (Amount found in mg)	**INTRA DAY** (Amount found in mg)
1.	9.92	10.13
2.	9.98	10.10
3.	9.86	10.10
4.	10.20	10.09
5.	10.00	10.20
6.	10.06	10.07
S.D	0.1182	0.0480
%RSD	1.1822	0.4748

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TABLE – 22

SYSTEM SUITABILITY TEST PARAMETERS

S.No.	Parameters	Values in Methanol : Phosphate Buffer (50:50)
1	Theoretical plates	3090
2	Tailing factor	1.45
3	Asymmetric factor	1.71
4	Capacity factor	0.96

TABLE – 23

OPTICAL CHARACTERISTICS OF ALFUZOSIN HC1 BY RP-HPLC METHOD

Parameters	Values
λ_{max}(nm)	245
Beers law limit (µg/ml)	1-5
Sandell's sensitivity (µg/cm^2/0.001 A.U)	6.8194E-10
Molar absorptivity (L mol^{-1} cm^{-1})	6.22363E+11
Correlation coefficient (r)	0.9995
Regression equation (y=mx+c)	Y=1465781.9X - 45649.76
Slope(m)	1465781.9
Intercept(c)	-45649.76

TABLE – 24

QUANTIFICATION OF FORMULATION –ALFOO

BY RP-HPLC METHOD

S.No	Labeled amount (mg/tab)	Amount found(mg)	Percentage obtained	Average %	SD	%RSD	SE
1.	10.00	9.86	98.60				
2.	10.00	10.06	100.60				
3.	10.00	10.09	100.90	99.18	1.2750	1.2855	0.0354
4.	10.00	9.80	98.00				
5.	10.00	9.80	98.00				
6.	10.00	9.90	99.00				

TABLE – 25

RECOVERY STUDIES FOR FORMULATION - ALFOO

BY RP-HPLC METHOD

S.No	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Estimated (µg/ml)	Amount Recovered (µg/ml)	% Recovery
1.	1.484	0.50	1.935	0.451	
2.	1.513	1.00	2.409	0.895	
3.	1.513	1.50	3.032	1.518	99.86
4.	1.453	2.00	3.351	1.897	
5.	1.451	2.50	3.962	2.511	
6.	1.484	3.00	4.385	2.901	

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