VALIDATED ANALYTICAL METHODS FOR THE SIMULTANEOUS ESTIMATION OF CEFPODOXIME PROXETIL AND AMBROXOL HYDROCHLORIDE BY UV SPECTROPHOTOMETRY AND RP-HPLC IN BULK AND TABLET DOSAGE FORM

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In partial fulfillment for the award of Degree of

MASTER OF PHARMACY

(Pharmaceutical Analysis)

Submitted by

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MAY-2012

CERTIFICATE

This is to certify that the research work entitled **"VALIDATED ANALYTICAL METHODS FOR THE SIMULTANEOUS ESTIMATION OF CEFPODOXIME PROXETIL AND AMBROXOL HYDROCHLORIDE BY UV SPECTROPHOTOMETRY AND RP-HPLC IN BULK AND TABLET DOSAGE FORM"** submitted to The Tamil Nadu Dr. M.G.R. Medical University in partial fulfillment for the award of the Degree of the MASTER OF PHARMACY (Pharmaceutical Analysis) was carried out by **BHAVYASRI. M (Register No. 26106121)** in the Department of Pharmaceutical Analysis under our direct guidance and supervision during the academic year 2011-12.

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Dedicated to

My

Family and

friends

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LIST OF ABBREVIATIONS

ICH	-	International Conference on Harmonisation
λ	-	Lambda
LOD	-	Limit of Detection
LOQ	-	Limit of Quantitation
↘g mL^{-1}	-	Microgram Per Millilitre
mg / tab	-	Milligram Per tablet
ml	-	Millilitre
nm	-	Nanometer
pH	-	Negative Logarithm of Hydrogen Ion
%	-	Percentage
% RSD	-	Percentage Relative Standard Deviation
HPLC	-	High Performance Liquid Chromatography
Rt or t_R	-	Retention Time
S.D	-	Standard Deviation
S.E	-	Standard Error
UV-VIS	-	Ultraviolet – Visible
AUC	-	Area under the curve
IR	-	Infra Red
°C	-	Degree Celsius
Gms	-	Grams
↘l	-	Microlitre
rpm	-	Rotations Per Minute
v/v	-	Volume / Volume
min	-	Minute
ml/min	-	Millilitre/Minute
HCl	-	Hydrochloric acid
CEF	-	Cefpodoxime proxetil
AMB	-	Ambroxol Hydrochloride

1.INTRODUCTION (www.DrugstoreTM.com)

Medicine is considered as one of major important necessity to all of us. It is derived from the Latin words as medicine meaning "the art of healing". It is a branch of health sciences and is the sector of public life concerned with maintaining or restoring human health through the study, diagnosis, treatment and possible prevention of disease, injury and other damage to the body or mind. It is both an area of knowledge, a science of body system and their diseases and treatment. This branch of science encompasses treatment by drugs, diet, exercise and other nonsurgical means. It is also used to maintain our health. An agent such as drug is used to treat disease or injury.

In the field of Pharmacology, potency is a measure of the drug activity expressed in terms of amount required to produce an effect of given intensity. A highly potent drug evokes a larger response at low concentrations, while a drug of lower potency evokes a small response at low concentrations. It is proportional to affinity and efficacy.

To demonstrate potency using an analytical assay as a surrogate measurement of biological activity, one should provide sufficient data to establish a correlation between the surrogate measurement(s) and the biological activity (ies) that is related to potency. The relationship between the surrogate measurement and biological activity may be established using various approaches, which includes comparison to preclinical/proof of concept data, in vivo animal or clinical data, or in vitro cellular or biochemical data. While choosing to use an analytical assay as a surrogate measurement of biological activity to meet the potency requirements for licensed biological products, you should meet criteria. The ability to measure potency is very

essential to product characterization; one should initiate potency assay development during preclinical and early clinical investigations to obtain as much product information as possible.

In addition, measuring drug potency during early product development has a number of advantages, such as:

- Demonstrate product activity, quality and consistency throughout product development
- Generate a collection of data to support specifications for lot release
- Provide a basis for assessing manufacturing changes
- Evaluate product stability
- Evaluate multiple assays
- Recognize technical problems or reasons a different assay might be preferable

Presently drug analysis and Pharmaceutical impurities are the subjects of constant review in the public interest. The International Conference of Harmonisation (ICH) guidelines achieved a great deal in harmonizing the definition of impurities in new drug substances. It is necessary to perform all the investigations on appropriate reference standards of drug and impurities to get meaningful specifications. In order to meet the challenges to ensure high degree of purity of drug substances and drug products a scheme is proposed for profiling drug impurity. Finally analytical methods based on analytical Instrumentation must be employed to quantitate drug substance and its impurities.

1. 1 ANALYTICAL CHEMISTRY *(Devala rao G., 2008; Khare R.P., 2007)*

Analytical chemistry may be defined as the science and art of determining the composition of materials in terms of the elements (or) compounds contained. In analytical chemistry it is prime importance to gain information about the qualitative and quantitative composition of substances and chemical species

Introduction to analytical methods brought a drastic change, in which physical property of a substance is measured to determine its chemical composition. An analysis instrument is a device (or) a set of devices that acquires the desired information regarding the chemical composition (or) the physical properties of a given sample (or) the process. This information may be required for a variety of purposes, eg: testing of materials, maintenance of standards, verification of physical phenomena, monitoring the process stream, controlling product quality safety management and so on. Analysis instrumentation is the science of technology of developing such measuring devices. Analytical chemistry including quantitative analysis is of enormous importance in science and industry. Chemical analysis is a most important method of investigation and it is widely used in all branches of sciences which are related to chemistry. At present no material is taken into production or released into the market without analytical data which characterize its quality and suitability for various purposes. Analysis of intermediate products is of enormous importance. The qualitative analysis gives us the information about the nature of sample by knowing about the presence or absence of certain components.

Aims and objectives of analytical chemistry *(kellner R., 2004)*

Analytical chemistry has two main aims (intrinsic and extrinsic). The intrinsic aim is the achievement of the metrological activity i.e ensuring full consistency between the analytical results delivered and the actual value of the measured parameters. The extrinsic aim is solving the analytical problems derived from the (bio)chemical information needs posed by a variety of 'clients'(eg private companies, social agents, research centers) or, in other words, providing client satisfaction. Broadly speaking the principle objective of the analytical chemistry is to obtain as much (bio)chemical information and of as high quality as possible from objectives and systems by using as little material, time human resources as possible and with minimal costs and risks.

APPLICATIONS OF ANALYTICAL CHEMISTRY TO VARIOUS BRANCHES

Analytical chemistry theory practice R.M. Verma

Analytical chemistry plays a very significant role in chemical research as every chemist uses directly data obtained by applying techniques. Apart from applications to chemical research, analytical techniques are frequently employed in industry in connection with problems such as, quality control and in ascertaining most appropriate experimental conditions for obtaining maximum yield of a particular product. It should be noted that techniques of analytical chemistry find wide application not only in different branches of chemistry but also in other physical and biological sciences and in many fields of engineering.

Geologists are analytical procedures for analyzing ground water, minerals, rocks, ores etc... In agriculture, chemical analysis is used to determine the composition of soils, in the production of fertilizers, insecticides and weed killers.

Medical and biological research programmes depend on chemical analysis which helps in developing medicines to cure various diseases.

In order to safeguard public health there is constant checking of foods, drugs, cosmetics, water supplies etc., and this is done in analytical laboratories. Waste disposals and the composition of air in industrial areas are analysed to know the extent of harm they would cause to public health, so that necessary preventive steps can be taken.

Analytical methods **(***P C Kamboj, 2003. Annees A. Siddiqui, 2006)*

The pharmaceutical analysis defined as "the branch of practical chemistry which deals with the resolution, separation, identification, determination and purification of a given sample of a medicine, the detection and estimation of impurities, which may be present in drug substance (or) given sample of medicine".

The substance may be a single compound or a mixture of compounds and may be in the form a tablet, pill, capsule, ampoule, liquid, mixture or an ointment.

The quality control tests involve methods which embrace chemicals, physio -chemical/ instrumental, microbiological (or) biological procedures.

The pharmaceutical analysis deals with the subject of determining the composition of material in terms of the elements or compound (drug) present in the system.

Any type of analysis involves two steps

Identification (qualitative)

Estimation (quantitative)

In qualitative analysis, a reaction is performed in such a way as to indicate the formation of a precipitate, a change of a colour, the dissolution of a precipitate/ complex formation and the evaluation of a gas.

Quantitative analysis is performed ordinarily through five steps. They are sampling, dissolution, precipitation, measurement and calculation.

Method of assay

It indicates the quantitative determination of principal ingredients of the official substances and in preparations.

Qualitative analysis

T his is practiced in order to establish the composition of a naturally occurring or artificially synthesized/ manufactured substance.

Qualitative analysis

I. Chemical Methods

a) Titrimetric analysis

b) Gravimetric analysis

c) Gasometric analysis

II. Physio - Chemical Methods (Instrumental Methods)

III. Microbiological Procedures

IV. Biological Procedures

I. Chemical Methods

a. Titrimetric Analysis

The analysis based on the fact that in all balanced chemical reactions utilized for the purpose. Equivalent weight of one substance reacts quantitatively with the equivalent weight of the other substance. The difference types of titration are as follows

Acid base titrations (neutralization titrations)

Non- aqueous titrations

Redox titrations (redox = oxidation - reduction)

Precipitation titrations

Complexometric titrations

b. Gravimetric Analysis

This method involves the conversion of the element or a radical to be determined into a pure stable compound readily convertible into a form suitable for weighing.

c. Gasometric Analysis

This type of analysis involves the measurement of the volume of gases. The volume of a gas set free in a given chemical reaction under the conditions similar to those described in the process. It may be noted that the volume of gas is taken at normal conditions and pressure or standard temperature and pressure (NTP/ STP) which is a temperature of 0^0C (273.09^o K) and the pressure of a column of 760mm/ Hg at 0^0C. If the reaction is taken place under different temperature and pressure the volume is adjusted to standard conditions. A decrease in the volume of gas when a suitable reagent is placed to absorb one of the gases present. This decrease in volume is also reduced to STP.

The gases cyclopropane, CO_2, NO_2, oxygen, octyl nitrite, Nitrogen, amyl nitrite, ethylene and helium are determined by gasometric analysis. The measurement of volume of gases is usually done by means of gas burettes or nitrometers.

II. Physio - Chemical Methods (Instrumental Methods)

Initially analytical methods were depending on extraction procedure, volumetric and gravimetric methods. All these methods are nearly replaced by advanced instrumental methods. These methods are more sensitive, specific and accurate but cost factors of the instruments and their maintenance are the main draw

backs. Various instrumental methods are classified depending on the property analyzed.

Shows different Instrumental methods with basic principle

Sr. N0.	**METHOD**	**BASIC PRINCIPLE**
A	ELECTROANALYTICAL METHODS	
1	Potentiometry	Concerned with change in electrical properties of the system measures the change in electrode potential during a chemical reaction of the system
2	Conductometry	Measures the change in electrical conductivity during a chemical reaction
3	Polarography	Measure the current at various applied potential indicating the polarization at indicator electrode
4	Amperometry	Measure the change (or decrease) in current at a fixed potential during addition of titrant
B	SPECTROSCOPIC METHODS	
1	Absorption Spectroscopy (Ultraviolet-Visible and Infrared)	Measure the absorbance or percent transmittance during the interaction of monochromatic radiation (or particular wavelength) by the same
2	Fluorimetry	Measure the intensity of fluorescence caused by emission of electromagnetic radiation due to absorption of UV radiation
3	Flame Photometry	Measure the intensity of emitted light of particular wave length emitted by particular element

4	Turbidimetry	Measure the turbidity of a system by passing light beam in a turbid media
5	Nephlometry	Measure the opalescence of the medium by reflection of light by a colloidal solution
6	Atomic Absorption Spectrometry	Measure the intensity of absorption when atoms absorbs the monochromatic radiation
7	X-Ray Spectroscopy	Measure the position and intensity of spectral lines during emission of X ray spectrum by atoms under influence of X rays
8	Refractometry	Measure the refractive index by causing refraction of light by matter
9	Polarimetry	Measure optical reaction by causing the rotation of plane polarized light
C	Mass Spectroscopy	Observe the position and intensity of signals in mass spectrum by causing the ionization of molecules
D	NMR Spectroscopy	Observe the position and intensity lines in NMR spectrum when proton interact with electromagnetic radiation in radio frequency region
E	Thermal Methods	Measure the physical parameters of the system as a function of temperature. It includes thermo gravimetry, derivative gravimetry, differential thermal analysis
F	Radiometric Methods	Measure the radioactivity either present naturally or induced artificially

III. Microbiological Methods

`In a microbiological assay, a comparison of inhibition of the growth of bacteria by a measured concentration of the antibiotic, which is to be examined, is made with that produced by known concentration of the standard preparation of an antibiotic having known activity.

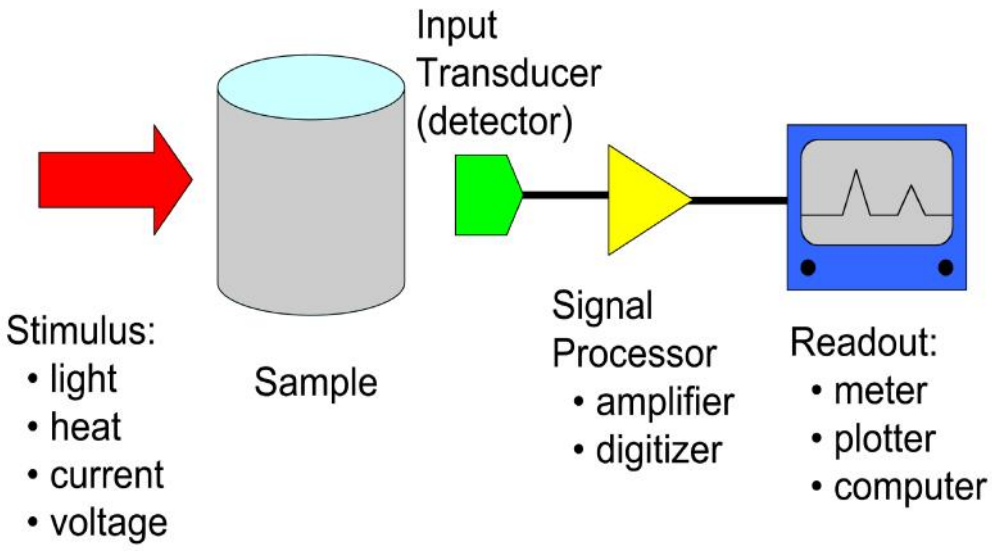
IV. Biological Methods

When the potency of a drug or its derivative cannot be properly determined by physical or chemical methods and where it is possible to observe the biological effects of the drug on some type of living matter. The biological assays are carried out. The basis of such assay is to determine how much of the sample gives the same biological effect as a given quantity of the standard preparation. The sample and standard preparation are tested under identical conditions in all respect. In a typical bio – assay, a stimulus is applied to a subject is referred to as the dose and is indicated by a weight or in terms of the concentration of the preparation. The application of stimulus on a subject produces some observable effect and this is called the response. The response may be measured by the total weight or weight of some organ of the subject, blood sugar concentration, and diameter of inhibition zone or by some other physiological symptoms.

1.2 ULTRAVIOLET SPECTROSCOPY (*Beckett A.H and stenlake J.B., 2002*)

Ultraviolet spectroscopy deals with the measurement of energy absorbed when electrons are promoted to higher energy state. On passing electromagnetic radiation in the ultraviolet and visible regions through the compound with multiple bonds, a portion of the radiation is normally absorbed by the compound. The amount of absorption depends on the wavelength of the radiation and the structure of the compound. Absorption of the electromagnetic radiation in the visible and ultraviolet region of spectrum results in changes of electronic structure of ions and molecules.

Diagram of an Analytical instrument showing the stimulus and measurement of response.



QUANTITATIVE SPECTROPHTOMETRIC METHODS

(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 photometer and spectrophotometric method are high selectivity and ease of convenience. Quantitative analysis (assay of an absorbing substance) can be done using following methods.

- Use of $A^{1\%}_{1\ cm}$ values
- Use of calibration graph (multiple standard method)
- By single or double point standardization method.

i) Use of $A^{1\%}_{1\ cm}$ values

This method can be used for estimation of drug from formulations or raw material, when reference standard not available. The use of standard value $A^{1\%}_{1\ cm}$ avoids the need to prepare a standard solution of the reference substance in order to determine its absorptivity, and is of advantage in situations where it is difficult or expensive to obtain a sample of the reference substance.

ii. Use of calibration graph

In this procedure the absorbances of a number (typically 4-6) of standard solutions of the reference substance at concentrations encompassing the sample concentrations are measured and a calibration graph is constructed. The concentration of the analyte in the sample solution is read from the graph as the concentration corresponding to the absorbance of the solution. Calibration data are essential if the absorbance has a non-linear relationship with concentration, or if the absorbance or linearity is dependent on the assay conditions. In certain visible spectrophotometric assays of colourless substances, based upon conversion to coloured derivatives by heating the substance with one or more reagents, slight variation of assay conditions, e.g. P^H, temperature and time of heating, may rise to a significant variation of absorbance, and experimentally derived calibration data are required for each set of samples.

iii. Single or double point standardization

The single point procedure involves the measurement of the absorbance of a sample solution and of a standard solution of the reference substance. 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.

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

Where,

C_{test} and C_{std} are the concentration in 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. 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.

Std1 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 are 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 and that of the solution in the reference (blank) cell.

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

- Simultaneous equation method
- Absorbance ratio method
- Geometric correction method
- Orthogonal polynomial method
- Difference spectrophotometry
- Derivative spectrophotometry
- Chemical derivatisation

1.2.1 Methods carried out

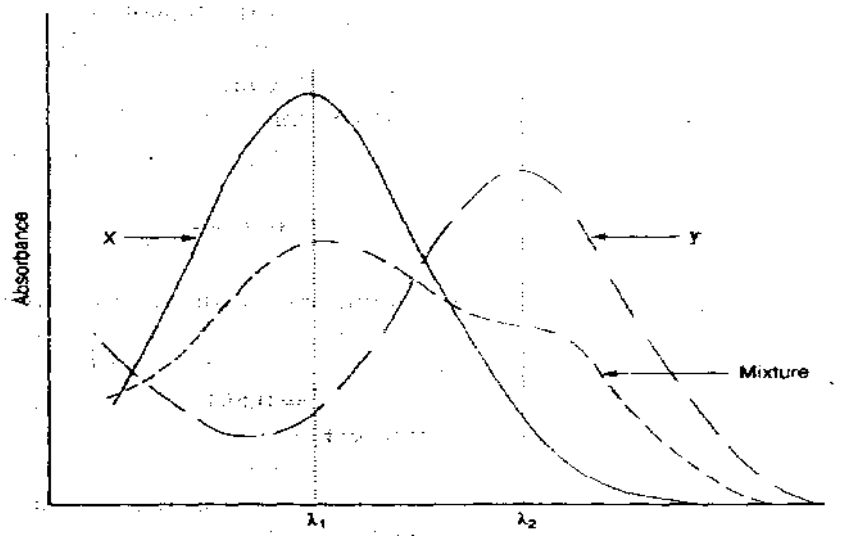
i. SIMULTANEOUS EQUATION METHOD

ii. AREA UNDER THE CURVE METHOD

iii. DERIVATIVE SPECTROSCOPIC METHOD

i. SIMULTANEOUS EQUATION METHOD

If a sample contains two absorbing drugs (X and Y) each of which absorbs at λ_{max} of the others it may be possible to determine both drugs by the technique of simultaneous equation (Vierodt's method) provided that criteria apply.



Information required is

1. The absorptivities of X at λ1 and λ2 are ax1 and ax2, respectively
2. The absorptivities of Y at λ1 and λ2 are ay1 and ay2, respectively
3. The absorbances of the diluted sample at λ1 and λ2, A1 and A2 respectively.

Let c_x and c_y be the concentrations of X and Y respectively in the diluted sample. Two equations are constructed based upon the fact that at λ1 and λ2

$$c_x = \frac{A_2 a_{y_1} - A_1 a_{y_2}}{a_{x_2} a_{y_1} - a_{x_1} a_{y_2}}$$

$$c_y = \frac{A_2 a_{x_2} - A_2 a_{x_1}}{a_{x_2} a_{y_1} - a_{x_1} a_{y_2}}$$

Criteria for obtaining maximum precision, based upon the absorbance ratios, have been suggested (Glenn, 1960) that place limits on the relative concentrations of the components of the mixture. The criteria are the ratios.

$$\frac{A_2 / A_1}{a_{x_2} / a_{x_1}} \text{ and } \frac{a_{y_2} / a_{y_1}}{A_2 / A_1}$$

Should lie outside the range 0.1-2.0 for the precise determination of X and Y respectively. These criteria are satisfied only when the λ_{max} of the two components is reasonably dissimilar. An additional criterion is that the two components do not interact chemically, there by negating the initial assumption that the total absorbance is equal to sum of the individual absorbances.

ii. AREA UNDER THE CURVE METHOD (*Telekone et al*., *2010*)

The area under curve method is applicable where there is no sharp peak or when broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ_1 and λ_2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area has to be calculated. This wavelength area is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. In combination drugs λ_1 and λ_2 denotes the wavelength ranges of the components. The integrated value of absorbance in the wavelength ranges of both the

drugs are substituted in the simultaneous equation to get the concentration of the drugs.

$$c_x = \frac{A_2 a_{y_1} - A_1 a_{y_2}}{a_{x_2} a_{y_1} - a_{x_1} a_{y_2}} \quad \text{And} \quad c_y = \frac{A_1 a_{x_2} - A_2 a_{x_1}}{a_{x_2} a_{y_1} - a_{x_1} a_{y_2}}$$

iii. DERIVATIVE SPECTROSCOPIC METHOD

This method involves the conversion of the normal spectrum into first, second or higher derivative spectrum. The transformation that occurs in the derivative spectrum is understood by reference to a Gaussian band which represents an ideal absorption band.

The first derivative (D^1) spectra is a plot of the ratio of change of absorbance with wavelength against wavelength, i.e a plot of slope of the fundamental spectrum against wavelength or a plot of $dA/d\lambda$ Vs λ_1. At λ_2 and λ_4, the maximum positive and maximum negative slope respectively in the D°. Spectrum corresponds with maximum and minimum respectively in the D^1 spectrum. The λ_{max} at $\lambda_3$is a wavelength of zero slope and gives $dA/d\lambda$, i.e a cross-over point, in the D1 spectrum.

The first order derivative spectrum of absorption band is characterized by a maximum, a minimum and a cross-over at a λ_{max} of the absorption band. These spectral transformations confer two main advantages on derivative spectrophotometry. Firstly an even order spectrum is of narrower spectral band width than its fundamental spectrum.

Derivative spectrum shows better resolution of overlapping bands than the fundamental spectrum and may permit the accurate determination of λ_{max} of the

individual bands. Secondly, derivative spectroscopy discriminates in favours of the substances of narrow spectral bandwidth against broad band width substances.

The absorption laws (Y.R. Sharma, 2009)

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

(1) Lambert's Law

(2) Beer's Law

Lambert's Law

When a beam of monochromatic radiation passes through a homogenous absorbing medium, the rate of decrease of intensity of radiation with thickness of absorbing medium is proportional to the intensity of incident radiation.

$$I = I_0 e^{-kt}$$

Where, I_0 = Intensity of incident light

I = Intensity of emerged light

t = Thickness of the medium

Beer's Law

When a beam of monochromatic radiation is passed through a solution of an absorbing substance, the rate of decrease of intensity of radiation with concentration of the absorbing solution is directly proportional to the intensity of incident radiation.

$$I = I_0 e^{-kc}$$

Where, I_0 = intensity of incident light

I = Intensity of emerged light

c = concentration of the absorbing species

From these laws, the following empirical expression of Beer - Lambert's Law was constructed

$$\text{Log } (I_0/I_T) = Єct = A$$

Where, A= Absorbance or optical density or extinction co-efficient

Є = Molecular extinction co-efficient

c = Concentration of drug

t = Path length

Limitations of Beer Lambert's Law

1. When different forms of the absorbing molecules are in equilibrium as in keto-enol tautomers.
2. When fluorescence compounds are present.
3. When solute and solvent forms complex through some sorts of association.

1.3 INTRODUCTION TO HPLC METHODS OF ANALYSIS OF DRUGS IN COMBINED DOSAGE FORM *(Chatwal R Gurdeep, et al., 2008)*

High performance liquid chromatography [HPLC] was developed in the late 1960's and 1970's it is widely accepted separation technique for both sample analysis and purification in a variety of areas including the pharmaceutical, biotechnological, environmental polymer and food industries.

HPLC instrumentation is made up of eight basic components they are mobile phase reservoir, solvent delivery system, sample introduction device, column, detector, waste reservoir, connective tubing and a computer, integrator (or) recorder.

Chromatography is defined as a method of separating a mixture of components into individual components through equilibrium distribution between two phases. Chromatography technique is based on the difference in the rate at which the components of a mixture move through a porous medium (stationary phase) under the influence of some solvent or gas (mobile phase).

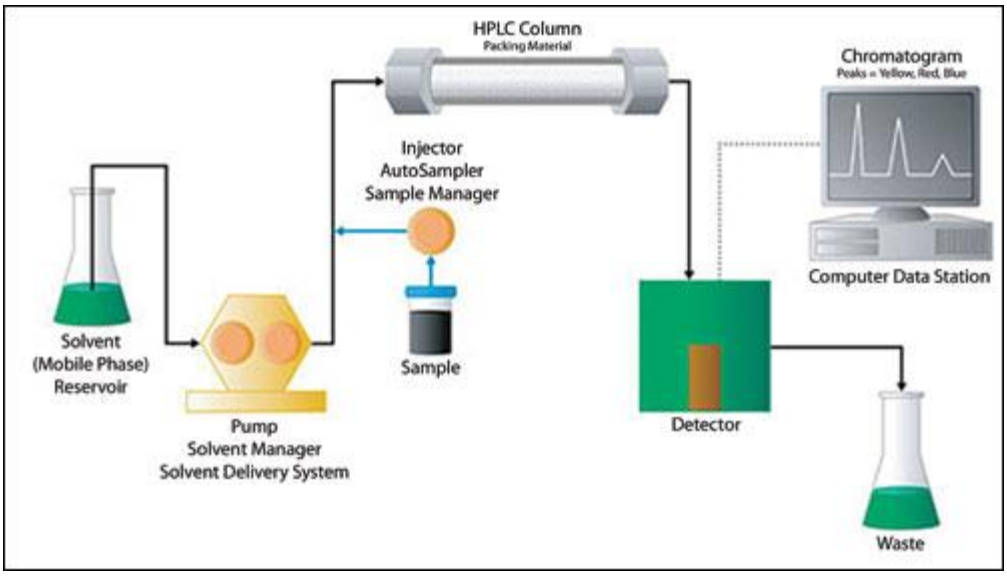
The chromatographic method of a separation in general involves the following steps:

- Adsorption or retention of a substance or substance on the stationary phase.
- Separation of the adsorbed substance by the mobile phase.
- Recovery of the separated substance by a continuous flow of the mobile phase. The method being called elution.
- Quantitative and qualitative analysis of the eluted substance

1.3.1 Introduction to HPLC *(Sharma B K., 2006)*

HPLC is a form of liquid chromatography to separate compounds that are dissolved in solution. HPLC instrument consists of four basic parts

- The column
- Detector
- Injection system
- Mobile-phase pump system



A schematic diagram of HPLC equipment

1.3.2 Principle of separation in HPLC *(Willard et al., 1986)*

The principle of separation in normal phase and reverse phase mode is the adsorption. When a mixture of components is introduced in to a HPLC column, they travel according to their relative affinities towards the stationary phase. The component which has more affinity towards the adsorbent travels slower. The components which have less affinity towards the stationary phase travel faster. Since

no two components have the same affinity towards the stationary phase the components are separated.

1.3.3 Modes of chromatography

i. Normal phase mode

ii. Reverse phase mode

i. Normal phase chromatography

In normal phase mode, the stationary phase (silica gel) is polar in nature and the mobile phase is non-polar. In this technique non-polar compounds travel faster and eluted first. The silica structure is saturated with silicon groups at the end and 'OH' groups attached to silicon atoms are the active binding sites.

ii. Reverse phase chromatography

In reverse phase technique, a non polar stationary phase is used. The mobile phase is polar in nature hence polar components get eluted first and non-polar compounds are retained for a longer time. Since most of the drugs and pharmaceutical are polar in nature, they are not retained for a longer and eluted faster, which is advantageous. Different columns used are ODS (octadecyl silane) or C_{18}, C_8 and C_4 etc.

1.4 ICH GUIDELINES FOR ANALYTICAL METHOD VALIDATION

(Code Q2A; Q2B. ICH Guidelines1994 and 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. The International Conference of Harmonization (ICH) of technical requirements for the registration of pharmaceutical for human use has developed a consensus text on validation of analytical procedures. The document includes definition for eight validation characterstics.

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 quantification
- ✓ Robustness
- ✓ Ruggedness

1.4.1 SPECIFICITY

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to present. An investigation of specificity should be conducted during the validation of identification tests, the determination of impurities and assay.

1.4.2 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.

1.4.2.1 Assay

- Assay of Active substances
- Assay of Medicinal products

Several methods are available to determine the accuracy

a) Application of an analytical procedure to an analyte of known purity
b) Comparision of the results of the proposed analytical procedure
c) Application of the analytical procedure to synthetic mixtures

1.4.2.2 Impurity (Quantification)

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

Eg: weight/weight or area percent

1.4.3 PRECISION

The precision of an analytical procedure expresses the closeness of the agreement between a series of measurements obtained from multiple sampling of same homogeneous sample under the prescribed conditions. Validation of tests for assay and for quantitative determination of impurities includes an investigation of precision.

1.4.3.1 Repeatability (intra- assay precision)

Express the precision under small operating conditions over a short interval of time. It should be assessed using a minimum of nine determinations.

1.4.3.2 Intermediate Precision

The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used. Typical validation to be studied includes days, analysts, equipments, etc.

1.4.3.3 Reproducibility

Reproducibility is assessed by means of an inter-laboratory trail. Reproducibility should be considered in case of the standardization of an analytical procedure, for insistence inclusion of procedure in pharmacopoeias.

1.4.4 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 sample.

1.4.5 RANGE

Range of an analytical procedure is the interval between the upper and lower concentration 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.

1.4.6 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.

a. Based on visual evaluation
b. Based on Signal-to-Noise ratio
c. Based on the standard deviation of the response and the slope
    - Based on the standard deviation of blank
    - Based on the calibration graph

The detection limit (DL) may be expressed as

$$DL = \frac{3.3\sigma}{S}$$

Where,

σ = standard deviation of the response

S= slope of the calibration curve (of the analyte)

1.4.7 LIMIT OF QUANTIFICATION

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

a. Based on visual evaluation

b. Based on Signal-to- Noise ratio

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

- Based on the standard deviation of blank
- Based on the calibration graph

The quantification limit may be expressed as,

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

Where,

σ = standard deviation of the response

S = slope of the calibration curve (of the analyte)

1.4.8 ROBUSTNESS

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

1.4.9 RUGGEDNESS

The united states of pharmacopoeia (USP) define ruggedness as the degree of reproducibility of test results obtained by the analysis of the same sample under a variety of normal test condition 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.

1.5 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 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 analysed constitute an integral system that can be evaluated as such. FDA guidelines on "validation of chromatographic methods" the following acceptance limits are proposed as initial criteria.

System suitability parameters and recommendations

S.No.	Parameters	Recommendations
1	Theoritical plates (N)	>2000
2	Tailing factor (T)	≤ 2
3	Assymetric factor	≤ 2
3	Resolution (Rs)	> 2 between peak of interest and the closest eluting potential interference
4	Repeatability	RSD ≤ 1% for N ≥5 is desirable
5	Capacity factor (k^1)	> 2.0
6	Relative retention	Not essential as long as the resolution is stated

1) Capacity Factor (or) Retention (K_A)

The retention of a drug with a given packing material and eluent can be expressed as retention time or retention volume, but both of these are dependent on flow rate, column length and column diameter. The retention is best described as a column capacity ratio (K), which is independent of these factors. The column capacity ratio of a compound (A) is given as

$$K_A = \frac{V_A - V_0}{V_0} = \frac{t_A - t_0}{t_0}$$

2) Resolution (R_S)

The resolution, R_s of two neighboring peaks is defined by the ratio of the distance between the two peak maxima. It is the difference between the retention times of two solutes divided by their average peak width. For baseline separation, the ideal value of R_s is 2.0. It is calculated by using the formula,

$$R_f = \frac{Rt_2 - Rt_1}{0.5\,(W_1 + W_2)}$$

Where,

Rt_1 and Rt_2 are the retention times of components 1 and 2

W_1 and W_2 are peak widths of components 1 and 2

3) Selectivity (⇨)

The selectivity (or separation factor) ⇨, is a measure of relative retention of two components in a mixture. The ideal value of selectivity is 2. It can be calculated by using the formula,

$$\alpha = \frac{V_2 - V_0}{V_1 - V_0}$$

Where, V_0 is the void volume of the column and V_2 and V_1 are the retention volumes of the second and the first peak, respectively.

4) Column efficiency

Efficiency, N, of a column is measured by the number of theoretical plates per meter. It is a measure of band spreading of a peak. Smaller the band spread, higher is the number of theoretical plates, indicating good column and system performance. Columns with N ranging from 5,000 to 1,00,000 plates/meter are ideal for a good system. Efficiency is calculated by using the formula,

$$N = 16 \frac{Rt^2}{W^2}$$

Where, Rt is the retention time and W is the peak width.

5) Peak asymmetry factor (A_s)

Peak asymmetry factor, A_s can be used as a criterion of column performance. The peak half width b of a peak at 10 % of the peak height, divided by the corresponding front half width a gives the asymmetry factor.

1.6 PHARMACEUTICAL STATISTICS

Linear regression

Linear regression a statistical technique that defines the functional relationship between two variables 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 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

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

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).

Standard deviation (SD)

It is commonly used in statistics as a measure of precision statistics 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

Where,

$$S = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{N - 1}}$$

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

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.

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

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

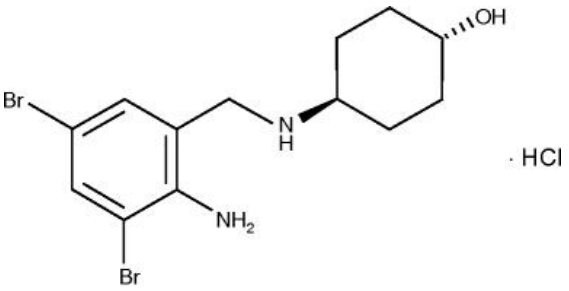
Where, S.D = Standard deviation = number of observations.

2. LITETRATURE REVIEW

2.1 DRUG PROFILE *(The Indian pharmacopoeia 2007)*

2.1.1 AMBROXOL HYDROCHLORIDE

Molecular structure



$C_{13}H_{19}Br_2ClN_2O$

Chemical name

trans-4-[(2-Amino-3,5-dibromobenzyl)amino] cyclohexanol hydrochloride.

Molecular formula

$C_{13}H_{18}Br_2N_2O.HCl$

Molecular weight

414.6

Category

Mucolytic expectorant**.**

Description

A white or yellow crystalline powder.

Solubility

Ambroxol HCl is sparignly soluble in water, soluble in methanol, practically insoluble in methylene chloride.

Storage

Protect from light. Following reconstitution, aliquot and freeze at -20^{o}C. This product is stable for 2 years as supplied. Stock solutions are stable for 4 months at -20^{0}C.

Identification

i) Melting point

Standard value	Observed average value*
233 ºC -236ºC	234.66ºC

***Average of six observations**

ii) Infra red spectrum

iii) Thin layer chromatography

Test solution – dissolve 50mg of the substance to be examined in methanol and dilute to 5ml with the same solvent.

Reference solution – dissolve 50mg of Ambroxol HCl in methanol & dilute to 5ml with the same solvent.

Plate – TLC silica gel F_{254} plate.

Mobile phase – con.ammonia, 1-propanol, ethyl acetate, hexane.

Drying in air.

Detection – examine in UV at 254nm.

Results – the principle spot in the chromatogram obtained with the test solution is similar in position and size to the principle spot in the chromatogram obtained with the reference solution.

$_{P}$H

4.5 -6

Mechanism of Action

The substance is a mucoactive drug with several properties including secretolytic and secretomotoric actions that restore the physiological clearance mechanisms of the respiratory tract which play an important role in the body's natural defense mechanisms. It stimulates synthesis and release of surfactant by type II pneumocytes. Surfactants acts as an anti-glue factor by reducing the adhesion of mucus to the bronchial wall, in improving its transport and in providing protection against infection and irritating agents. Ambroxol HCl enhances penetration power of antibiotics. Administration of Ambroxol together with antibiotics leads to higher antibiotic concentration in the lung tissue. It also act as a scavenger of hypochlorous and hydroxyl radicals, it blocks nitric oxide stimulated activation of guanylate cyclise.

Contraindications

Ambroxol should not be used in patients known to be hypersensitive to Ambroxol or other components of the formulation

Ambroxol side effects

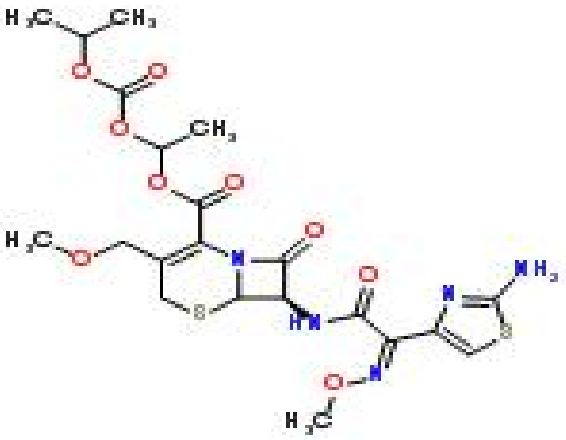
Occasional gastro intestinal side effects may occur but these are normally mild.

Overdosage

No symptoms of overdosage have been reported in man to date. If they occur, symptomatic treatment should be provided. Interactions Administration of Ambroxol together with antibiotics (amoxicilline, cefuroxime, erythromycin, doxycycline) leads to higher antibiotic concentration in the lung tissue. No clinically relevant unfavorable interaction with other medications has been reported.

2.1.2 CEFPODOXIME PROXETIL

Molecular structure *(The Merck index 2006, 14th edition)*



Chemical name

[6R-[6α,7β(z)]]-7-[[(2-amino-4-thiozolyl)(methoxyimino)acetyl]amino]-3-(methoxy-methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylicacid1-[[(1-methyl ethoxy)carbonyl]oxy]ethyl ester.

Molecular formula

$C_{21}H_{27}N_5O_9S_2$

Molecular weight

557.61

Category

Antibiotic:Used for treating respiratory track infection and urinary track infection.

Description

It is white to light brownish powder,odourless or bitter in taste**.**

Storage

Store at not exceeding 25ºC, Protect in tight containers**.**

Solubility

Freely soluble in methanol and ethanol, slightly soluble in ether, very slightly soluble in water and practically insoluble in chloroform.

Identification

i) **Melting point**

Standard value	Observed average value*
111 ºC - 113ºC	111ºC

***Average of six observations**

ii) **Infra red spectrum**

Mechanism of action

It is a orally administered THIRD generation cephalosporin's class of antibacterial agent more active against enterobacteriaceae,streptococcus aureus and β-lactamase producing H.influenzae,M.catarrhalis,N.gonorrhoeae & less active against gram +ve cocci. It binds to one or more of the penicillin binding proteins (PBPs) which inhibits the final transpeptidation step of peptidoglycon synthesis in bacterial cell wall, thus inhibiting biosynthesis and arresting cell wall assembly resulting in bacterial cell death.

Absorption

Decreased absorption in conditions of low gastric acidity. Bioavailability 50%.

Half life 2.2hrs.

Distribution

Cefpodoxime proxetil distributed in respiratory and GU tract in therapeutic concentration.

Enters in breast milk in low concentrations.

Protein binding 20-30 %.

Interactions

Antacids or H2 blockers may decrease the absorption of Cefpodoxime. Probenecid inhibits renal secretion. Monitor renal function during administration. Additive nephrotoxic effects with furosemide.

2.2 REPORTED METHODS

REPORTED METHODS FOR AMBROXOL HYDROCHLORIDE

1. Umadevi. B *et al*., **(2011),** reported "**Development and Validation of UV Spectrophotometric determination of Doxofylline and Ambroxol HCl in bulk and combined tablet formulation**". The method employs simultaneous equation using the absorbance at 274 and 244.5nm for Doxofylline and Ambroxol. For absorbance correction method 274nm for Doxofylline and 308nm for Ambroxol were Doxofylline shows nil absorbance.

2. Nagavalli. D *et al*., **(2011),** reported "**Validated HPLC method for the Simultaneous estimation of Gemifloxacin Mesylate and Ambroxol HCl in bulk and tablet dosage form**". The method has been developed with mobile phase acetonitrile, methanol and trifluro acetic acid at the ratio of (25:20:55 % v/v) detected in 248nm observed retention time were 2.69 mins and 3.43 mins.

3. Jain P.S.1 *et al*., **(2010)**, reported "**Stability-Indicating HPTLC determination of Ambroxol Hydrochloride in bulk drug and pharmaceutical dosage form**". The method employed HPTLC aluminium plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of methanol-triethylamine (4:6, v/v). Densitometric analysis of Ambroxol hydrochloride was carried out in the absorbance mode at 254 nm.

4. Prathap. B *et al*., **(2010),** reported "**Simultaneous determination of Gatifloxacin and Ambroxol Hydrochloride from tablet dosage form using RP-HPLC".** A Reversed-Phase High Performance Liquid Chromatography (HPLC) method was developed, validated, and used for the quantitative determination of Gatifloxacin (GA) and Ambroxol Hydrochloride (AM), from its tablet dosage form.

Chromatographic separation was performed on a Thermo Hypersil Keystone ODS C18 column (250 mm × 4.6 mm, 5 µm), with a mobile phase comprising of a mixture of phosphate buffer and acetonitrile (60:40, v/v), and pH adjusted to 3 with ortho phosphoric acid, at a flow rate of 1 mL/min, with detection at 250 nm.

5. Deshpande *et al*., **(2010),** reported "**Application of HPLC and HPTLC for the Simultaneous determination of Cefixime Trihydrate and Ambroxol HCl in pharmaceutical dosage form**". The HPTLC method involves desitometric measurements at 254nm, the separation was on aluminium sheets of Silica gel 60 F 254 using acetonitrile : methanol : triethylamine (8.2:1:0.8,v/v/v) as mobile phase. The HPLC method was carried on column C18 at ambient temperature using mobile phase acetonitrile: methanol (50:50, v/v) UV detection at 254nm.

6. Senthil Raja. M *et al*., (**2010),** reported **"RP-HPLC method Development and validation for the simultaneous estimation of Azithromycin and Ambroxol Hydrochloride in Tablets".** The separation was carried out using a mobile phase consisting of acetonitrile and mono basic potassium phosphate buffer of pH 8.5 in the ratio of 65:35 v/v. The column used was C18 phenomenex Gemini 5m, 250cm x 4.6mm id with flow rate of 2ml/min using PDA detection at 220nm.

7. Makarand Avhad *et al*., **(2009),** reported "**Develoment and validation of Simultaneous UV- spectrophotometric method for the determination of Levofloxacin and Ambroxol in tablets**". The method involves Q-absorbance equation at 219nm isoabsorptive point and at 287nm using distilled water as a solvent.

8. Krishna Veni Nagappan *et al*., **(2008)**, reported "**A RP-HPLC Method for Simultaneous Estimation of Ambroxol Hydrochloride and Loratidine in Pharmaceutical Formulation".** The method was carried out on a Phenomenex

Gemini C18 (25 cm x 4.6 mm i.d., 5 µ) column with a mobile phase consisting of acetonitrile: 50mM Ammonium Acetate (50:50 v/v) at a flow rate of 1.0 mL/min. Detection was carried out at 255 nm.

9. Neela M Bhatia *et al*., **(2008)**, reported "**RP-HPLC and Spectrophotometric estimation of Ambroxol and Cetirizine Hydrochloride in combined dosage form**". The chromatographic methods were standardized using a HIQ SIL-C 18 column (250×4.6 mm i.d., 10 µm particle size) with UV detection at 229 nm and mobile phase consisting of methanol-acetonitrile-water (40:40:20, v/v/v).

10. Lakshmana prabhu. S *et al.,* **(2008)**, reported "**Simultaneous UV spectrophotometric estimation of Ambroxol HCl and levoceterizine Dihydrochloride**". The method involved solving simultaneous equations based on measurement of absorbance at two wavelengths 242nm and 231nm.

11. Pai PNS *et al.,* **(2006)**, reported "**Determination of Ambroxol Hydrochloride using Dithiocarbamic acid Colorimetric method".** A new simple, colorimetric method was developed on the basis of a chemical reaction of amine group in Ambroxol Hydrochloride with carbon disulphide to form Dithiocarbamic acid, which on further reaction with cupric chloride forms a colored copper chelate. The yellowish-orange chromophore has absorption maxima of 448 nm.

12. Meiling Qi *et al.,* **(2004)**, reported "**Liquid chromatography method for determination of Roxithromycin and Ambroxol Hydrochloride in a new tablet formulation**". This chromatographic method was achieved on a Diamonsil TM C18 column. The mobile phase consisting of a mixture of acetonitrile, methanol and 0.5% ammonium acetate (39:11:50v/v) Detection was carried out at 220nm.

13. Dincer *et al.,* **(2003)**, reported "**Quantitative determination of Ambroxol in tablets by Derivative UV spectrophotometric method and HPLC**". Determination of Ambroxol was conducted by using First-order derivative UV-spectrophotometric method at 255nm.This chromatographic method was achieved on C_{18} column with a mixture of aqueous phosphate (0.01 m), acetonitrile and glacial acetic acid (59:40:1, v/v/v).

14. Kuchekar. B.S *et al*., (2003) reported "**Spectrophotometric estimation of Ambroxol HCl in tablets**". The colorimetric method was carried out by two different reagents by using Sodium nitrite, Napthyl ethylene diamine produced pinkish red chromogen at 500nm and by using Ferric nitrate and Nitric acid produced yellowish orange chromogen at 400nm.

15. Francisco G *et al.,* **(2001)**, reported "**Determination of Ambroxol Hydrochloride by HPLC**". Reverse phase liquid chromatography was employed, using methanol-0.01 M di ammonium phosphate buffer, pH=6, (70:30, v/v) and a detector wavelength of 247 nm**.**

16. Narayana reddy. M *et al.,* **(1998),** reported "**Spectrophotometric determination of Ambroxol**". The method developed by using reagents 3-methyl-2-benzolinone hydrazone (MBTH) and Ferric chloride ($FeCl_3$) and Potassium ferricyanide [$K_3Fe(CN)_6$].

REPORTED METHODS FOR CEFPODOXIME PROXETIL

17. Shah. M. N *et al.,* **(2012),** reported "**Development and Validation of Spectophotometric method for Simultaneous determination of Cefpodoxime proxetil and Ofloxacin in tablets**". The method involved solving Simultaneous equations based on measurement of absorbance at two wavelengths 236 and 299nm in methanol.

18. Patel Sanket A *et al.,* **(2011),** reported "**Simultaneous Spectrophotometric determination of Cefpodoxime proxetil and Ofloxacin in tablets**". This method involves the ratio of absorbances at two selected wavelength one isoabsorptive point at 273.2nm and the other 297nm for Cefpodoxime proxetil and Ofloxacin in methanol.

19. Patel Sanket A *et al.,* **(2011),** reported "**Dual wavelength spectrophotometric method for Simultaneous estimation of Ofloxacin and Cefpodoxime proxetil in tablet dosage form**". This method was based on determination of Ofloxacin at the absorbance difference between 224nm and 247.4nm, for Cefpodoxime proxetil the absorbance difference is between 278.2nm and 320nm.

20. Patel Sanket A *et al.,* **(2011),** reported "**Development and Validation of First order Derivative spectrophotometric method for simultaneous estimation of Ofloxacin and Cefpodoxime proxetil in tablet dosage form**". This method involves the simultaneous determination of Ofloxacin and Cefpodoxime proxetil by derivative spectrophotometric method at zero crossing points 236.4nm for Cefpodoxime proxetil and 208.8nm for Ofloxacin.

21. Sunil singh *et al.,* **(2010),** reported "**Spectrophotometric and RP-HPLC methods for simultaneous determination of Cefpodoxime proxetil and Clavunate**

Potassium in combined tablet dosage form". This method involves the Simultaneous equation estimation 0f Cefpodoxime proxetil and Clavunate potassium at 235nm and 270nm in methanol.

22. Malathi. S *et al.,* **(2009),** reported "**Simultaneous Rp-HPLC estimation of Cefpodoxime proxetil and clavunic acid in tablets**". This method was carried out on a Zorbax Eclipse XDB 5µ C18 (150 x 4.6mm) column with mobile phase consisting of acetonitrile:50 mm potassium dihydrogen phosphate buffer (pH 3.0) at the ratio of 70:30 v/v at detection 228nm.

23. Gandhi.S.V *et al.,* **(2009),** reported "**Simultaneous spectrophotometric determination of Cefpodoxime proxetil and Potassium clavunate**". This method employs absorbance correction method at the wavelength 288nm for CEF were Potassium clavunate shows nil absorbance its absorbance was observed at 218nm. And for derivative spectroscopy 235.6nm and 300.2nm was selected for Potassium clavunate and CEF respectively.

24. Darji. S. H *et al.,* **(2007),** reported "**Development and Validation of HPTLC method for the estimation of Cefpodoxime proxetil**". The proposed method was validated by using mobile phase a mixture of Chloroform:Methanol:Toluene (4:2:4 v/v/v). The detection spot was carried out at 289nm.

25. Shah. P. B *et al.,* **(2006),** reported "**RP-HPLC for the determination of Cefpodoxime proxetil**". In this method the drug was eluted from a 5mcm reverse phase column at 25ºC temperature with mobile phase consisting of 50mm Phosphate buffer (pH 3.0 ± 0.1) adjusted with 10% phosphoric acid and methanol (80:20,v/v) with detection at 228nm.

26. Srinivasa rao. Y *et al.,* **(2003),** reported "**Spectrophotometric method for the estimation of Cefpodoxime proxetil**". This method is based on the reaction of the drug with Ferric chloride and Potassium ferricyanide, which forms a green chromogen exhibiting maximum absorption at 780 nm.

3. AIM AND PLAN OF WORK

The combined dosage form selected for the present study containing Cefpodoxime proxetil and Ambroxol HCl in tablets, this combination recently entered into the market. Cefpodoxime proxetil and Ambroxol HCl in combined dosage form used in treatment of respiratory tract infections.

In the view of the literature cited for the quantification of Cefpodoxime proxetil and Ambroxol HCl, it was found that the method for estimation of Cefpodoxime proxetil and Ambroxol HCl individually and in combination with other drugs were available. No method available for the simultaneous estimation of Cefpodoxime proxetil and Ambroxol HCl in combined dosage form.

Hence in the present work, the aim is to develop a simple, precise and accurate methods for the estimation of Cefpodoxime proxetil and Ambroxol HCl in bulk and in combined pharmaceutical dosage form and to validate the developed methods by UV spectrophotometry and RP – HPLC.

Validation of Developed method

The developed method should be validated as per ICH guidelines. The parameters used to validate the developed method are; Accuracy, Precision, Linearity, Range, Repeatability, Reproducibility, Limit of Detection, Limit of Quantification, Ruggedness. The system suitability parameters like Capacity factor, Asymmetry factor, Tailing factor, Theoretical plates, HETP and Resolution should be calculated for RP – HPLC chromatograms and compared with standard values.

For UV method:

1) Find the solubility of drugs in various solvents
2) To determine maximum absorbance and selection of wavelengths for detection.
3) Determining the standard absorbance for all selected wavelengths for each drug.
4) Development of simple, precise, accurate and sensitive
    - Simultaneous equation method
    - Area under the curve method
    - Derivative spectroscopic method , in the specified range
5) Validation of developed methods as per ICH guidelines.

For RP-HPLC method:

1) Selection of suitable mobile phase for two drugs with proper resolution and short duration of time
2) Development of chromatogram with various concentration for each drug to determine range of concentrations
3) Relating area of chromatogram with respect to concentration for individual drugs
4) Determination of percentage purity of physical mixture and in formulation
5) Validation of the developed method

4. MATERIALS AND METHODS

4.1 MATERIALS

4.1.1 Drugs

Cefpodoxime proxetil and Ambroxol HCl were generously gifted by Madras pharmaceuticals ltd Chennai and the formulation Finecef- AM TAB from a local pharmacy.

4.1.2 Reagents and Chemicals

All the chemicals used were of analytical grade and HPLC grade procured from Qualigens, India Ltd. The chemicals used for the study were

Methanol (analytical grade)

Acetonitrile (HPLC Grade)

Methanol (HPLC Grade)

Water (HPLC Grade)

Ortho phosphoric acid (Analytical Grade)

4.1.3 Instruments specifications

a) Shimadzu AUX- 200 digital balance
b) Shimadzu 1700 double beam UV-visible spectrophotometer with a pair of 10 mm matched quartz cells
c) Thermo scientific spectra HPLC system (INERTSIL ODS-3V)

d) Elico SL- 210 double beam UV-visible spectrophotometer with a pair of matched quartz cells.

e) Remi centrifuge apparatus

f) Sonicator model 2120 MH

g) Cyberlab micropipette

h) Elico LI 120 pH meter

i) Melting point apparatus - Guna enterprises Chennai

4.1.4 Specifications (Terms) of instruments

a) Shimadzu AUX – 200 digital balance: (Shimadzu instruction manual)

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

b) Shimadzu UV – Visible spectrophotometer: (Shimadzu instruction manual)

Model : Shimadzu, UV-1700, pharmaspec.; Cuvetts: 1 cm matched 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 10 g/l) 0.04% or less (340 nm: $NaNo_2$ 50 g/l)
Measurement wavelength range	190~1100 nm
Spectral Band Width	1 nm or less (190 to 900 nm)
Wavelength Accuracy	± 0.5 nm automatic wavelength calibration mechanism
Recording range	Absorbance : -3.99~3.99 Abs Transmittance : -399~399%
Photometric Accuracy	± 0.004 Abs (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) Thermo scientific spectra High Performance Liquid Chromatography:

1. Column : Inertsil ods-3v (150mm X 4.6mm; 5 microns)

2. Column temperature : 30ºC

3. Sample cooler temperature : 10ºC

4. Pump: P 4000

5. Auto sampler : AS 3000

6. UV-Vis Detector : UV 2000

7. Vacuum Degasser : SCM 1000

8. System Controller : SN 4000

9. Software : Chrom Quest 5.0

4.2 METHODS EMPLOYED

The methods employed for simultaneous estimation of Cefpodoxime proxetil and Ambroxol HCl in combination are

4.2.1 UV Spectrophotometric method

a. Simultaneous equation method
b. Area under the curve method
c. Derivative spectroscopic method

Selection of solvent

The solubility of drugs was determined in a variety of solvents as per Indian pharmacopoeia standards. Solubility was carried out in polar to non polar solvents. The common solvent was found to be methanol for the analysis of Cefpodoxime proxetil and Ambroxol HCl for proposed method.

Preparation of standard stock solution

10 mg of Cefpodoxime proxetil and Ambroxol HCl raw material were weighed and transferred into 10 ml volumetric flasks separately and dissolved in methanol and made up to the volume with methanol. These solutions were observed to contain 1000 ↘g mL^{-1}.

Selection of wavelengths for estimation and stability studies

The selection of wavelengths for the estimation of Cefpodoxime proxetil and Ambroxol HCl a suitable diluted stock solution contain 10 ↘g mL^{-1} of each and the solutions were scanned between 200 – 400 nm by using methanol as blank. From the overlaid spectra, by the observation of spectral characteristics of Cefpodoxime proxetil and Ambroxol HCl were simultaneously estimated by Simultaneous equation Method, Area under the curve method and Derivative spectroscopic method. The wavelengths selected for simultaneous equation method were 235nm and 248 nm also 235nm and 308nm. For Area under the curve method was 229-238 nm and 291- 316nm. For Derivative Spectroscopic method, the zero order spectrum was derivatised to first order spectrum in that 235 nm was selected for the estimation of Ambroxol HCl, which is zero crossing for Cefpodoxime proxetil and 279nm was selected for the estimation of Cefpodoxime proxetil which is zero crossing for Ambroxol HCl. The Stability was performed by measuring the absorbance of same solution at different time intervals. It was observed that Cefpodoxime proxetil was stable for 3 hours and Ambroxol HCl is stable for more than 3 days at all the selected wavelengths.

Preparation of calibration graph

The aliquots of stock solution of Cefpodoxime proxetil (1-5 ml of 50 ↘g mL^{-1}) and Ambroxol HCl (1-5 ml of 30 ↘g mL^{-1}) were transferred into 10 ml volumetric flasks and made up to the volume to get 5-30µg/ml and 3-18µg/ml concentrations with methanol. The absorbance of different concentration solutions were measured at 235,248,308 nm in the normal spectrum and 229-238,291-316 nm in area under the curve and in 10-60µg/ml and 6-36µg/ml concentrations of CEF & AMB was taken 279,235 nm in the first derivative spectrum for Cefpodoxime proxetil and Ambroxol HCl. The calibration curve was plotted at their corresponding wavelengths. All two drugs Cefpodoxime proxetil and Ambroxol HCl were linear with the concentration range of 5-30 ↘g /ml and 3-18 ↘g/ml & 10-60µg/ml and 6-36µg/ml respectively at their respective wavelengths and thus, it obeys the Beer's law.

Quantification of formulation

Twenty tablets of formulation (**Finecef-AM TAB**) (containing Cefpodoxime proxetil eq. to Cefpodoxime 100mg and 60 mg of Ambroxol HCl) were weighed accurately. The average weight of tablets was found and powdered. The tablet powder equivalent to 30 mg of Ambroxol was weighed and transferred into 50 ml volumetric flask added a minimum quantity of methanol to dissolve the substance by using ultra sonication for 15 minutes and made up to the volume with the same (1000 ↘g mL^{-1}). The content filtered through Whatmann filter paper No. 41. From the clear solution, further dilutions were made by diluting 1 ml to 10 ml volumetric flask, further dilute 1 ml to 10 ml to obtain 10 ↘g/ ml of cefpodoxime which contains 6 ↘g/ml of Ambroxol HCl theoretically. The absorbance measurements were made 6 times for the formulation at 235 nm, 248 nm, and 308nm, in simultaneous equation method. For

area under the curve method the absorbance were 229-238 nm and 291-316 nm. And for First order derivative method it was measured at 279nm, 239nm and the absorbtivity values were calculated. From the absorptivity values of Cefpodoxime proxetil and Ambroxol HCl at 235 nm, 248 nm, 308 nm, 229-238 nm and 291-316 nm and 235nm, 279nm the amount of Cefpodoxime proxetil and Ambroxol HCl were determined by using Simultaneous equation method, Area under the curve method and Derivative spectroscopic method. The procedure was repeated for three times for each percentage.

Recovery studies

The recovery experiment was done by adding known concentrations of Cefpodoxime proxetil and Ambroxol HCl raw material to the 50% preanalyzed formulation. Standard Cefpodoxime proxetil and Ambroxol HCl in the range of 80 %, 100 % and 120% to the 50% preanalyzed formulation into a series of 10 ml volumetric flasks and dissolved with methanol and made up to the mark with the same. The contents were sonicated for 15 minutes. After sonication the solutions were filtered through Whatmann filter paper No. 41. The absorbances of the resulting solutions were measured at their selected wavelengths for determination of Cefpodoxime proxetil and Ambroxol HCl respectively. The amount of each drug recovered from the formulation was calculated for all the drugs by Simultaneous equation method, Area under the curve method and Derivative spectroscopic method. The procedure was repeated for three times for each percentage recovery.

Validation of developed method

Linearity

A calibration curve was plotted between concentration and absorbance. Cefpodoxime proxetil was linear with the concentration range of 5-30 ↘g /ml at 235nm, 248nm, 308 nm, 229-238 nm, 291-316 nm and 10-60µg/ml at 279nm and Ambroxol HCl showed the linearity in the range of 1-5 ↘g /ml at 235nm, 248 nm, 308 nm, 229-238 nm, 291-316 nm and 6-36µg/ml at 235 nm by obeying Beer's law.

Accuracy (Recovery studies)

Accuracy of the method was confirmed by recovery studies. To the 50% pre-analyzed formulation, a known quantity of raw materials of Cefpodoxime proxetil and Ambroxol HCl were added and the procedure was followed as per the analysis of formulation. The amount of each drug recovered was calculated. This procedure was repeated for three times for each concentration. The % RSD was calculated.

Precision

The repeatability of the method was confirmed by the analysis of formulation was repeated for 6 times with the same concentration. The amount of each drug present in the tablet formulation was calculated. The % RSD was calculated. The intermediate precision of the method was confirmed by intraday and inter day analysis i.e. the analysis of formulation was repeated three times in the same day and on three successive days. The amount of drugs was determined and % RSD also calculated.

Ruggedness

Ruggedness of the method was confirmed by the analysis of formulation was done by the different analysts. The amount and % RSD were calculated.

LOD and LOQ

The linearity study was carried out for six times. The LOD and LOQ were calculated by using average value of slope and standard deviation of response (Intercept).

4.2.2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD

Chromatographic method depends up on the nature of the sample, molecular weight and solubility. The drug selected for the present study was polar compound; hence it can be separated either by normal phase or reverse phase chromatography. Reverse phase chromatographic technique was selected for initial separations with the knowledge of properties of compound, C_{18} column was chosen as stationary phase and various mixtures of water pH – 5 with ortho phosphoric acid, methanol and acetonitrile were considered as mobile phase.

OPTIMIZATION OF MOBILE PHASE

MOBILE PHASE	RATIO	RETENTION TIME	
		CEF	AMB
AcN:MeOH:50mmPhos buffer (pH 3)	50:20:30	2.72 Bp	1.73,1.84 Sg
AcN:MeOH:50mmPhos buffer (pH 3)	40:20:40	4.28,4.54 Sg	1.95 Sp
AcN:MeOH:50mmPhos buffer (pH 3)	30:20:50	9.46,10.7 Sg	2.57 Sp
AcN:MeOH:50mmPhos buffer (pH 3)	40:10:50	6.66,7.26 Sg	1.98 Sp
AcN:MeOH:50mmPhos buffer (pH 3)	20:10:70	15 Bp	7.79 Tg
AcN:MeOH:20mmAcet buffer (pH 3)	30:50:20	2.24 Sp	3.18 Sp
AcN:MeOH:20mmAcet buffer (pH 3)	40:20:40	2.32 Sp	3.20Sp

AcN:MeOH:Water (pH3)	40:40:20	2.17 Bp	1.50 Sp
AcN:MeOH:Water (pH5)	40:40:20	2.22 Sp	3.82 Bp
AcN:MeOH:Water (pH6)	40:40:20	1.97 Sp	3.01 Sp
AcN:MeOH:Water (pH5)	25:50:25	2.56 Sp	5.51,6.03 Sg
AcN:MeOH:Water (pH5)	25:55:20	2.25 Sp	4.51,4.84 Sg
AcN:MeOH:Water (pH5)	30:40:25	2.47 Sp	5.29,5.61 Sg
AcN:MeOH:Water (pH5)	35:45:20	2.01 Sp	3.55 Sp
AcN:MeOH:Water (pH5)	30:50:20	3.45 Sp	4.30 Sp

Sg-splitting, Tg-tailing, Bp-Broad peak, Sp-Sharp peak.

AcN-Acetonitrile, MeOH-methanol, Phos-Phosphate, Acet-Acetate.

Selection of mobile phase and ↙max

Different mixtures of mobile phase with different ratios were selected and their chromatograms were recorded. From this Acetonitrile: Methanol: water pH – 5 with ortho phosphoric acid (30:50:20 % V/V/V) was selected as mobile phase, since these two drugs were eluted with sharp peak and with better resolution. Hence this mobile phase was used to optimize the chromatographic conditions.

The detection wavelength was measured by scanning the 10 ↘g/ml solution of Cefpodoxime proxetil and Ambroxol HCl in mobile phase, in UV- spectrophotometry, overlaid spectra and the wavelength of maximum absorption was selected as 247 nm.

Optimized Chromatographic Conditions

The following parameters were used for RP-HPLC analysis of Cefpodoxime proxetil and Ambroxol HCl.

Mode of operation - Isocratic

Stationary phase	- C_{18} column (150mm × 4.6 mm I, d., 5m)
Mobile phase	- Acetonitrile: Methanol: water pH – 5 with ortho phosphoric acid (30:50:20% v/v/v)
Detection wavelength	- 247 nm
Flow rate	- 1 ml / min
Temperature	- Ambient
Sample volume	- 20 µl
Operating pressure	- 121 kgf

Preparation of the Standard stock solution

Standard Cefpodoxime proxetil stock solution

Weighed accurately Cefpodoxime proxetil equivalent to cefpodoxime and transferred into a 100 ml standard volumetric flask separately and dissolved with minimum quantity of HPLC grade methanol and the volume was made up to the mark with HPLC grade methanol to get the concentration of 1000 ↘g mL^{-1} of Cefpodoxime proxetil.

Standard Ambroxol HCl stock solution

Weighed accurately 60 mg of Ambroxol HCl and transferred into a 100 ml standard volumetric flask separately and dissolved with minimum quantity of HPLC grade methanol and the volume was made up to the mark with HPLC grade methanol to get the concentration of 600 ↘g mL^{-1} for Ambroxol HCl.

Linearity and Calibration

From the standard solution, pipetted out 7-13 ml into a series of six 100 ml volumetric flask and made up to the mark with mobile phase to obtain the concentration range from 70-130 ↘g mL^{-1} of Cefpodoxime proxetil and 42-78 ↘g mL^{-1} of Ambroxol HCl solution were injected and chromatogram was recorded. The calibration curve was plotted between concentration verses peak area.

Quantification of Cefpodoxime proxetil and Ambroxol HCl

Twenty Tablets containing Cefpodoxime proxetil eq. to Cefpodoxime 100 mg and 60 mg of Ambroxol HCl were accurately weighed. Weighed the content of drug equivalent to 48mg, 60 mg, 72mg of Ambroxol HCl was transferred to a 100 ml volumetric flask and dissolved in HPLC grade methanol and sonicated for 15 minutes. The final concentration was 600 ↘g mL^{-1}. The above solution was filtered through whatmann filter paper and the clear solution was collected, 10 ml was pipetted into a 100 ml volumetric flask and made up to the mark with the mobile phase to produce 60 ↘g mL^{-1} solutions. The peak area measurements were done by injecting each sample three times and the amount of Cefpodoxime proxetil and Ambroxol HCl of lower, middle and higher concentrations were calculated from their respective calibration curve.

Recovery Studies

To ensure the reliability of the method, recovery studies were carried out by mixing a known quantity of standard drug solution with the pre-analyzed sample formulation and the content were mixed and made to the volume with mobile phase and Pre-analyzed by the proposed method, the percentage recovery was calculated.

Limit of Detection and Limit of Quantification

Preparation of calibration curve for the serial dilution of standard was repeated for six times. The limit of detection and limit of quantification of each were calculated by using the average value of slope and standard deviation of response (Intercept).

System Suitability Studies

The system suitability studies were carried out as specified in I.P. the parameter like Column efficiency, Tailing factor, Asymmetric factor, and Theoretical plate number and were calculated.

5. RESULTS AND DISCUSSION

Simultaneous estimation of multiple drug formulations have advantage that the methods were less time consuming and usage of solvent is minimized. Three simple, rapid, precise and accurate spectrophotometric and an isocratic RP – HPLC methods were developed and validated for the estimation of Cefpodoxime proxetil and Ambroxol HCl in mixture and in combined tablet dosage form. The drugs were identified by IR Spectroscopy given in the figure 1 and 2. The methods employed for the analysis of Cefpodoxime proxetil and Ambroxol HCl were

UV-spectrophotometric methods

Three simple, precise and accurate methods namely

Simultaneous equation method

Area under the curve method

Derivative spectroscopic method

RP- HPLC METHOD

5.1. SIMULTANEOUS EQUATION METHOD

The solubility of Cefpodoxime proxetil and Ambroxol HCl was determined as per Indian Pharmacopoeia. The numeral polar and non – polar solvents were tried to dissolve the drugs. From the solubility profile methanol was chosen as a common solvent for the estimation of Cefpodoxime proxetil and Ambroxol HCl. The solubility data is in Table-1 for Cefpodoxime proxetil and Ambroxol HCl.

The standard solutions of 10 ↘g $mL^{-1}$of Cefpodoxime proxetil and Ambroxol HCl in methanol were prepared individually and the solutions were scanned in

UV region in the wavelength range from 200 to 400 nm by using methanol as blank.

The individual and overlaid spectra of Cefpodoxime proxetil and Ambroxol HCl were recorded as shown in Figure 3, 4 and 5. From the spectrum, 235 nm was λ_{max} of Cefpodoxime proxetil and 248 nm also 308nm was λ_{max} of Ambroxol HCl, these two wavelengths used for Simultaneous estimation of Cefpodoxime proxetil and Ambroxol HCl respectively. Different aliquots of Cefpodoxime proxetil in Methanol were prepared in the concentration range of 5– 30 ↘g/ml. The absorbances of solutions were measured at 235 nm, 248nm and 308 nm. The calibration curve was plotted using concentration against absorbance. The calibration graph at 235nm, 248nm and 308 nm is shown in Figure-6, 7 and 8. Different aliquots of Ambroxol HCl in Methanol were prepared in the concentration range of 3 – 18 ↘g/ml. The absorbances of these solutions were measured at 235nm, 248 nm and 308nm. The calibration graphs were plotted and are shown in Figure 9, 10 and 11. The preparation of calibration curve was repeated for six times for each drug at their selective wavelengths. The optical parameters like, Sandell's sensitivity, Molar absorptivity, correlation coefficient, slope, intercept, LOD, LOQ and Standard error were calculated. The correlation coefficient for the two drugs was found to be above 0.999. This indicates that all the drugs obey Beer's law in the selected concentration range. Hence the concentrations were found to be linear. The optical characteristics of two drugs at their selective wavelengths are shown in Table-2 for Cefpodoxime proxetil and Table-3 for Ambroxol HCl.

FINECEF- AM TAB containing Cefpodoxime proxetil eq. to Cefpodoxime 100mg and 60mg of Ambroxol HCl was selected for analysis. The nominal concentration of Cefpodoxime proxetil from linearity (10 ↘g/ml) was prepared and also contains (6 ↘g/ml) Ambroxol HCl; the absorbances of the solution were

measured at their respective wavelengths. The percentage label claim present in tablet formulation was given in Table-4, 5 for Cefpodoxime proxetil and Ambroxol HCl, respectively.

The amount present in tablet formulation was in good concord with the label claim and the % RSD values were found to be 0.089032 and 0.160045 at 235,248nm and 0.080327 and 0.302204 at 235,308nm for Cefpodoxime proxetil and Ambroxol HCl, respectively. The low % RSD values indicate that the method has good precision. The results of analysis are shown in Table-4, 5.

Further the precision of the method was confirmed by Intraday and Inter day analysis. The analysis of formulation was carried out for three times in the same day and one time in the three consecutive days. The % RSD value of intraday and inter day analysis were found to be and 0.020845 and 0.582865 at 235,248nm and 0.017043 and 0.948845 at 235,308nm for Cefpodoxime proxetil, 0.07554 and 0.398516 at 235, 248 nm and 0.091992 and 0.168291 at 235,308nm for Ambroxol HCl. The results of analysis are shown in Table-6, 7. The results showed that the precision of the method was confirmed.

The developed method was validated for Ruggedness. It refers to the specific of one lab to multiple days which may include multiple analysts, multiple instruments and different sources of reagents and so on. In the present work it was confirmed by different analysts. The % RSD value by analyst 1 and analyst 2 were found to be 0.089032, 0.070458 at 235,248nm and 0.080327, 0.080456 at 235,308nm for Cefpodoxime proxetil and 0.160045, 0.191136 at 235,248nm and 0.302204, 0.302295 at 235,308nm for Ambroxol HCl respectively. The low % RSD

values indicate that the developed method was more rugged. The results are shown in Table 8, 9.

The accuracy of the method was performed by recovery studies. To the preanalysed formulation, a known quantity of Cefpodoxime proxetil and Ambroxol HCl raw material solutions were added at different levels. The absorbance of the solutions was measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 100.17 – 100.44 % at 235,248nm and 98.91- 99.62 % at 235, 308nm for Cefpodoxime proxetil and 98.97 – 99.70% at 235, 248nm and 100.26-100.49 % at 235, 308nm for Ambroxol HCl. The low % RSD value for the two drugs indicates that this method is very accurate. The recovery data is shown in Table- 10, 11.

5.2. AREA UNDER THE CURVE METHOD

A simple, accurate, rapid and precise Area under the curve method was developed and validated. Methanol was chosen as a common solvent for estimation of Ambroxol HCl and Cefpodoxime proxetil.

From the working standard solutions 6 ↘g mL^{-1} of Ambroxol HCl and 10 ↘g mL^{-1} Cefpodoxime proxetil in methanol was prepared separately and the solutions were scanned in UV region in the wavelength range from 200 to 400 nm by using Methanol as blank and the area was fixed between two nm. It is shown in the fig-12 and 13 respectively. From the individual spectra area absorbance was calculated between two nm were the absorbances was nearly same was selected for the estimation of Cefpodoxime proxetil and Ambroxol HCl simultaneously at 229-238nm, 291-316nm.

Different aliquots of Ambroxol HCl and Cefpodoxime proxetil were diluted to the concentration range separately in distilled water. The area of each solution curves were measured between 229-238nm and 291- 316 nm. The calibration curve was plotted using absorbance against concentration. The calibration graph at 229-238 nm and 291-316 nm for Cefpodoxime proxetil is shown in Figures 14- 15. The calibration graph at 229-238 nm and 291-316nm for Ambroxol HCl are shown figure-16, 17 respectively. The preparation of calibration curve was repeated for six times for each drug at their selective wavelengths. The optical parameters like, sandell's sensitivity, molar absorptivity, correlation coefficient, slope, intercept, LOD, LOQ and standard error were calculated. The correlation coefficient for both the drugs was found to be above 0.999. This indicates that both the drugs obey Beer's law in the selected concentration range. Hence the concentrations were found to be linear. The optical characteristics of both drugs at selected wavelengths are shown in Table-12 for Cefpodoxime proxetil and Table-13 for Ambroxol HCl

FINECEF-AM TAB each containing Cefpodoxime proxetil eq. to Cefpodoxime proxetil 100mg and 60mg of Ambroxol HCl was selected for analysis. The nominal concentration of Cefpodoxime proxetil from linearity 10 ↘g mL^{-1}, when prepared contains 6 ↘g mL^{-1} of Ambroxol HCl, the absorbance of the solutions were measured at their selected wavelengths. The amount present in tablet formulation was in good concord with the label claim and the % RSD values were found to be 0.071185 and 0.151326 for Cefpodoxime proxetil and Ambroxol HCl respectively. The low % RSD values indicate that the method has good precision. The result of the formulation estimations was shown in Table-14.

Further the precision of the method was confirmed by intraday and inter day studies. The analysis of formulation was carried out for three times in the same day and one time in the three consecutive days. The % RSD value of intraday and inter day analysis were found to be 0.017369 and 0.030643 for Cefpodoxime proxetil, 0.034482 and 0.066243 for Ambroxol HCl. The results of analysis are shown in Table-15. The results showed that the precision of the method was high.

The developed method was validated for ruggedness. In the present work it was confirmed by different analysts. The % RSD value by analyst 1 and 2 were found to be 0.071185 and 0.060199 for Cefpodoxime proxetil, 0.151326 and 0.103921 for Ambroxol HCl. The low % RSD values indicate that the developed method was more rugged. The results are shown in Table-16.

The accuracy of the method was confirmed by recovery studies. To the pre-analyzed formulation, a known quantity of mixture of Ambroxol HCl and Cefpodoxime proxetil raw material solutions were added at different levels. The absorbances of the solutions were measured at selected wavelengths and the percentage recovery was calculated. The percentage recovery was found to be 100.4674 % for Cefpodoxime proxetil and 99.3191 % for Ambroxol HCl. The % RSD values were found to be 0.029872 and 0.269187 for Cefpodoxime proxetil and Ambroxol HCl respectively. The low % RSD value for both drugs indicates that this method is very accurate. The recovery data is shown in Table-17.

5.3 DERIVATIVE SPECTROSCOPIC METHOD

A simple, accurate, rapid and precise first order derivative method was developed and validated. The common solvent used for estimation of Cefpodoxime proxetil and Ambroxol HCl was chosen is Methanol.

The sample solutions of 10 ↘g mL^{-1} of Cefpodoxime proxetil and Ambroxol HCl in methanol were prepared individually and the solutions were scanned in UV region in the wavelength range from 200 to 400 nm by using methanol as blank. The zero order spectrum were derivatised into first order derivative spectrum individually as shown in the figure- 18, 19. The overlaid first order derivative spectrum of Cefpodoxime proxetil and Ambroxol HCl was recorded as shown in Figure-20. From the spectrum, 235 nm and 279 nm were selected for the estimation of Cefpodoxime proxetil and Ambroxol HCl respectively without any interference. At 235 measured nm, Cefpodoxime proxetil was zero. At 279 nm, Ambroxol HCl has zero absorbance value. Hence these two wavelengths were selected for the analysis of Cefpodoxime proxetil and Ambroxol HCl, respectively. Different aliquots of Cefpodoxime proxetil and Ambroxol HCl were prepared in the concentration range of 10 - 60 ↘g/ml and 6 – 36 ↘g/ml, respectively. The absorbances of these solutions were measured at 235 nm and 279 nm in the first order derivative spectrum for Cefpodoxime proxetil and Ambroxol HCl, respectively. The plotted graphs are shown in Figure 21 and 22 for Cefpodoxime proxetil and Ambroxol HCl, respectively. The preparation of calibration curve was repeated for six times for each drug at their selective wavelength. The calibration curve was plotted using concentration against absorbance. The optical parameters like, Sandell's sensitivity, Molar absorptivity, Correlation coefficient, Slope, Intercept, LOD, LOQ and Standard error were calculated for the two drugs. The correlation coefficient for the two drugs was found to be above 0.999. This indicates that the two drugs obey Beer's law in the selected concentration range. Hence the concentrations were found to be linear. The results are shown in Table-18.

FINECEF- AM TAB containing Cefpodoxime proxetil eq. to Cefpodoxime proxetil 100mg and 60mg of Ambroxol HCl was selected for analysis. The solution contains 10 ↘g mL^{-1} of Cefpodoxime proxetil was prepared (nominal concentration in the calibration curve of Cefpodoxime proxetil), which also contains 6 ↘g mL^{-1} of Ambroxol HCl, the absorbance of these solutions were measured at 279 nm and 235 nm. The amount of six test solutions was determined. The amount present in tablet formulation was in good concord with the label claim and the % RSD values were found to be 0.406486 and 0.595885 for Cefpodoxime proxetil and Ambroxol HCl, respectively. The results of analysis are shown in Table-19. The low % RSD values indicate that the method has good precision.

Further the precision of the method was confirmed by Intraday and Inter day analysis. The analysis of formulation was carried out for three times in the same day and one time in the three consecutive days. The % RSD value of Intraday and Inter day analysis are 0.368593 and 0.198141 for Cefpodoxime proxetil, 0.635013 and 0.664226 for Ambroxol HCl, respectively. The results of analysis are shown in Table-20. Hence the precision was confirmed. The results showed that the precision of the method was further confirmed.

The developed method was validated for Ruggedness. It refers to the specific of one lab to multiple days which may include multiple analysts, multiple instruments and different sources of reagents and so on. In the present work it was confirmed by different analysts. The % RSD value by analyst 1 and analyst 2 were found to be 0.406486 and 0.230238 for Cefpodoxime proxetil and 0.595885 and 0.893351 for Ambroxol HCl, respectively. The low % RSD values indicate that the developed method was more rugged. The results are shown in Table-21.

The accuracy of the method was performed by recovery studies. To the pre-analyzed formulation, a known quantity of Cefpodoxime proxetil and Ambroxol HCl raw material solutions were added at different levels. The absorbance of the solutions was measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 100.86-101.18 % for Cefpodoxime proxetil, and 99.84-100.29 % for Ambroxol HCl. The low % RSD value for the two drugs indicates that this method is very accurate. The recovery data is shown in Table 22.

5.4 RP-HPLC METHOD

An involvement was made in this project to device, a simple, accurate, less expensive and sensitive RP-HPLC method of estimation of Cefpodoxime proxetil and Ambroxol HCl in solid dosage form. Since the drug is polar reverse phase high performance liquid chromatography was selected.

Selection of Mobile Phase

Acetonitrile was preferred because of its lower viscosity and high UV transparency. Methanol was selected due to its inexpensiveness and also it is a good diluent for this drug. Acetonitrile: Methanol: Water in different ratio was tried and elution of was founded as splitted. Hence choice of orthophosphoric acid was incorporated to adjust pH. According to the pKa values of both the drugs pH-3 and pH-5 were selected. An attempt was made in phosphate buffer instead of water in different ratio, this also gave splitted peaks. Hence Acetonitrile: Methanol: Water pH-5 in the ratio 30:50:20 v/v/v this gave sharper peaks and it was selected as mobile phase.

The detection wavelength was measured by scanning the solution of Cefpodoxime proxetil and Ambroxol HCl in mobile phase. In UV-spectrophotometry, spectra was overlined and wavelength of maximum absorption was selected as 247 nm.

The limit of detection and the limit of quantification were determined by using slope and standard deviation and it was calculated. The system suitability parameters such as Theoretical plate, Tailing factor, Asymmetric factor and Resolution were calculated and shown in Table-23, the parameters were found to be satisfactory as per ICH guidelines.

With the optimized chromatographic conditions, stock solutions of Cefpodoxime proxetil and Ambroxol HCl were prepared in HPLC methanol and the final dilution with mobile phase and prepared the mixture in the concentration range 70-130 ↘g mL^{-1} of Cefpodoxime proxetil and 42-78 ↘g mL^{-1} of Ambroxol HCl, 20 µl of each solution was injected and records the chromatogram at 247 nm.

The chromatogram optimized given in Figure-26, the calibration curve was plotted using concentration against peak area from the fig-27 to fig-33. The procedure was repeated for three times. The correlation coefficient was found to be above 0.9997 and 0.9999 Cefpodoxime proxetil and Ambroxol HCl. The calibration graph of Cefpodoxime proxetil and Ambroxol HCl are shown in Fig-34 and Fig-35 respectively. The optical characteristics of Cefpodoxime proxetil and Ambroxol HCl shown in Table-24.

The Tablet dosage form FINECEF-AM TAB was selected for the analysis. The ostensible concentration 80 ↘g mL^{-1} , 100 ↘g mL^{-1} , 120 ↘g mL^{-1} of Cefpodoxime proxetil in the mobile phase were prepared which contains 48 ↘g mL^{-1} , 60 ↘g mL^{-1} ,

72 ↘g mL^{-1} of Ambroxol HCl. 20 µl of each solution was injected and chromatograms were recorded. The percentage purity was found to be 99.18%, 99.27%, 99.15% of low, middle, and high level dilutions for Cefpodoxime proxetil and 99.85%, 99.90%, 99.52% of low, middle, and high Ambroxol HCl respectively.

The precision of the method was confirmed by repeatability of formulation for three times of each level dilutions and the chromatograms are shown in Fig 36-44. The % RSD was found to be 0.2100, 0.1400 and 0.0903 of three levels for Cefpodoxime proxetil and 0.0251, 0.01423, 0.1045 Ambroxol HCl respectively. The data is shown in Table-25.

The accuracy of the method was performed by recovery studies to the pre analysed formulation, a known quantity of Cefpodoxime proxetil and Ambroxol HCl working standard solutions were added at different levels, injected the solutions. The chromatograms were recorded as shown in the Fig 45-47. The percentage recovery was found to in the range between 99.29-100.65% for Cefpodoxime proxetil and 98.99-100.38% for Ambroxol HCl. The percentage RSD was found to be 0.6885 and 0.7006 for Cefpodoxime proxetil and Ambroxol HCl respectively. The low percentage of RSD values for recovery indicated that the method was found to be accurate. The values given in the Table-26, the high percentage recovery revealed that no interference was produced due to the excipients used in formulation. Therefore developed method was found to be accurate.

All the above parameters with the ease of operations ensure that the projected methods could be applied for the routine analysis of Cefpodoxime proxetil and Ambroxol HCl pure form and in tablet dosage form.

6. SUMMARY AND CONCLUSION

Cefpodoxime proxetil and Ambroxol HCl in combination used in treatment of lower respiratory tract infections. A rapid, precise and accurate analytical method for the simultaneous estimation of Cefpodoxime proxetil and Ambroxol HCl in formulation (FINECEF- AM TAB) was developed.

FINECEF- AM TAB has been selected for study, which containing Cefpodoxime proxetil eq. to Cefpodoxime 100 mg and 60 mg of Ambroxol HCl.

The methods proposed for this combination of drugs are

6.1 UV SPECTROSCOPIC METHOD:

UV spectrophotometric method for the estimation of Cefpodoxime proxetil and Ambroxol HCl in combined dosage form by

(1) Simultaneous equation method

(2) Area under the curve method

(3) Derivative spectroscopic method

From the solubility data methanol is used as common solvent. Cefpodoxime proxetil and Ambroxol HCl were prepared separately (10 ↘g mL^{-1}) and scanned in UV region. From the overlaid spectra, by the observation of spectral characteristics of Cefpodoxime proxetil and Ambroxol HCl, they were selected for Simultaneous estimation, Area under the curve method and Derivative spectroscopic method. The wavelengths selected for simultaneous estimation method were 235nm, 248 nm and 308nm, 229-238nm and 291-316 nm for the area under the curve method and 279 nm and 235 nm for the Derivative spectroscopic method.

1. Simultaneous Equation Method

The percentage label claim present in tablet formulation **(FINECEF- AM TAB)** was found to be 99.87% and 101.09 % at 235,248nm and 101.64% and 99.58% at 235, 308nm for Cefpodoxime proxetil and Ambroxol HCl respectively. The percentage recovery was found to be in the range of 100.17 – 100.44% and 98.97- 99.70% at 235, 248nm and 98.91-99.62% and 100.26-100.49% at 235,308nm for Cefpodoxime proxetil and Ambroxol HCl.

In this accuracy, precision of two wavelengths of Ambroxol was compared. In which 248nm is précised one than 308nm studied by its optical characteristics obtained from linearity was good.

2. Area under the curve Method

The percentage label claim present in tablet formulation **(FINECEF- AM TAB)** was found to be 99.73% and 100.48% for Cefpodoxime proxetil and Ambroxol HCl respectively. The percentage recovery was found to be in the range of 100.44 – 100.50% and 99.03- 99.55% for Cefpodoxime proxetil and Ambroxol HCl.

3. Derivative Spectroscopic Method

The percentage label claim present in tablet formulation **(FINECEF- AM TAB)** was found to be 99.94% and 101.77% for Cefpodoxime proxetil and Ambroxol HCl respectively. The percentage recovery was found to be in the range of 100.86 –101.23 % and 99.84- 100.29% for Cefpodoxime proxetil and Ambroxol HCl.

6.2 RP – HPLC METHOD:

RP-HPLC method has been developed for the estimation of both drugs in bulk and in formulation. The proposed method gives reliable assay results with short analysis time, using mobile phase Acetonitrile: Methanol: water pH-5 with orthophosphoric acid in the ratio of 30:50:20 v/v/v. The percentage purity present in tablet formulation **(FINECEF-AM TAB)** for 80%, 100%, 120% was found to be 99.18 %, 99.27% and 99.15% for Cefpodoxime proxetil and 99.85%, 99.90%, 99.52% Ambroxol HCl repectively. The percentage recovery was found to be in the range of 99.29 –100.65 % and 98.99- 100.38 % for Cefpodoxime proxetil and Ambroxol HCl. The contents of drug present in the formulation were found to be satisfactory and system suitability parameters are in desired limit.

All the above methods do not suffer from any interference due to common excipients. It indicates that methods were accurate. Therefore the proposed methods could be successfully applied to estimate commercial pharmaceutical products containing Cefpodoxime proxetil and Ambroxol HCl.

Thus the above study's findings would be helpful to the analytical chemists to apply the analytical methods for the routine analysis of the analyte in pharmaceutical dosage forms.

Figure-1

IR SPECTRUM OF CEFPODOXIME PROXETIL

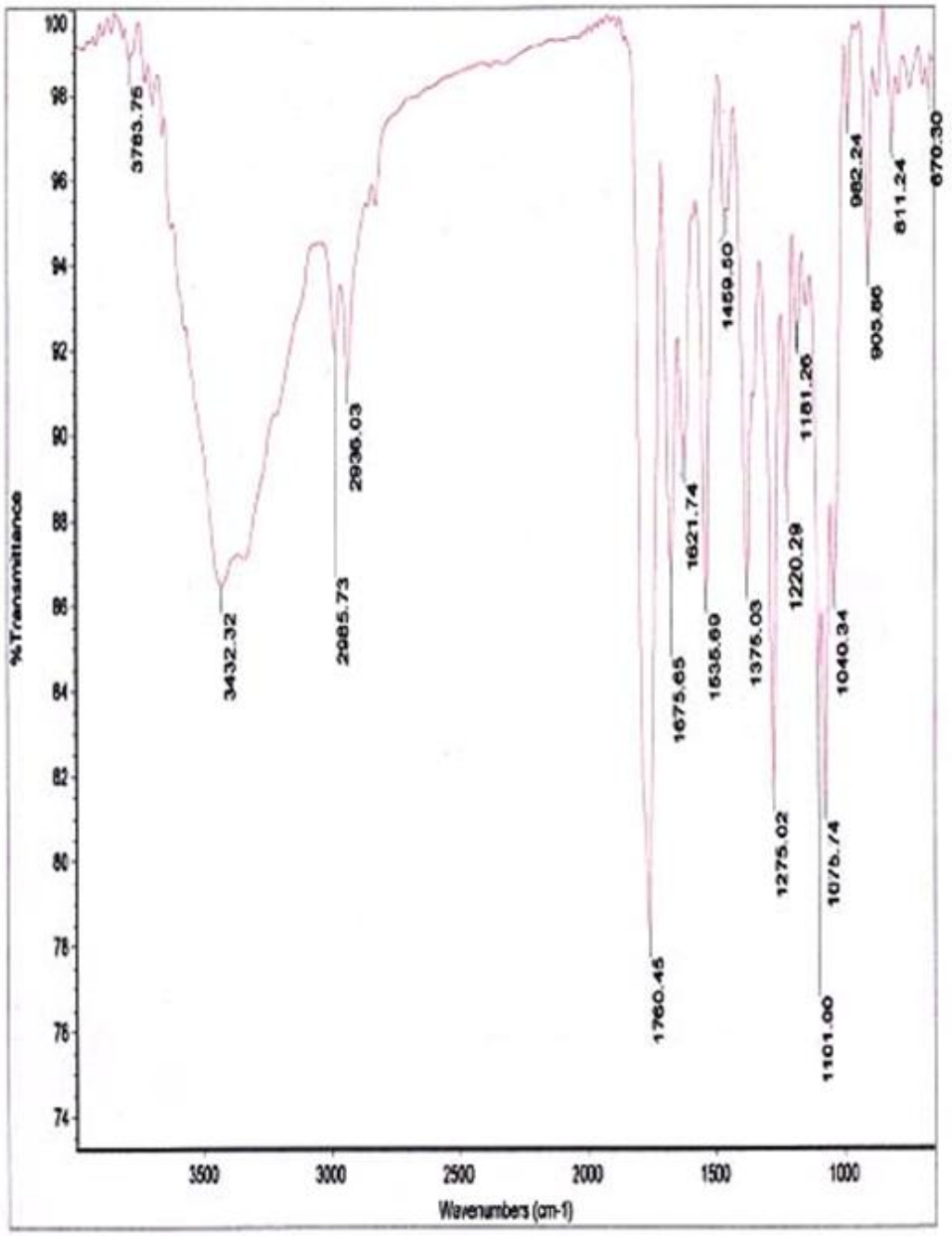


Figure-2

IR SPECTRUM OF AMBROXOL HYDROCHLORIDE

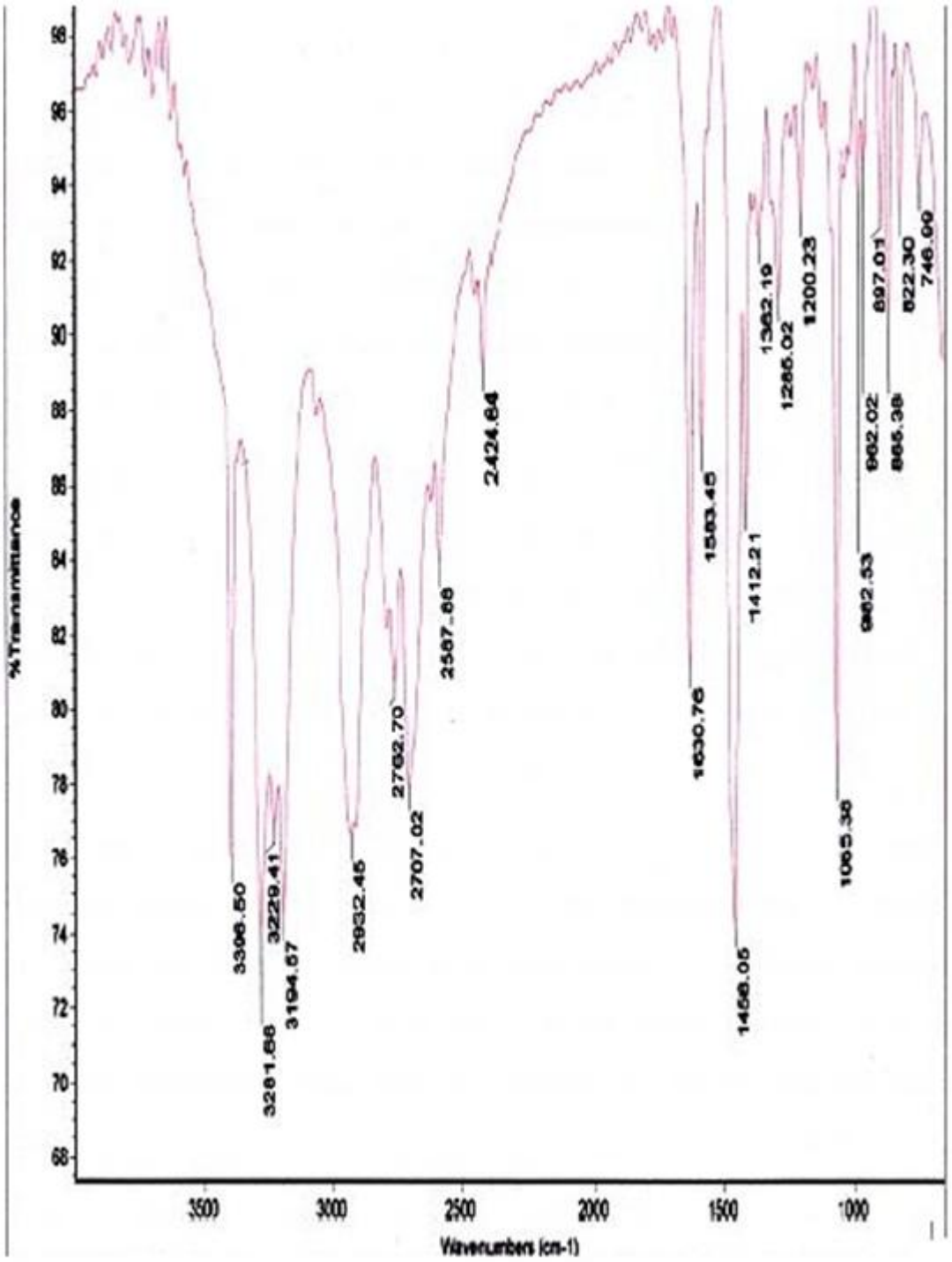


Figure-3

UV SPECTRUM OF CEFPODOXIME PROXETIL IN METHANOL

AT 235 nm (SIMULTANEOUS EQUATION METHOD)

Concentration: 10 ↘g mL^{-1}

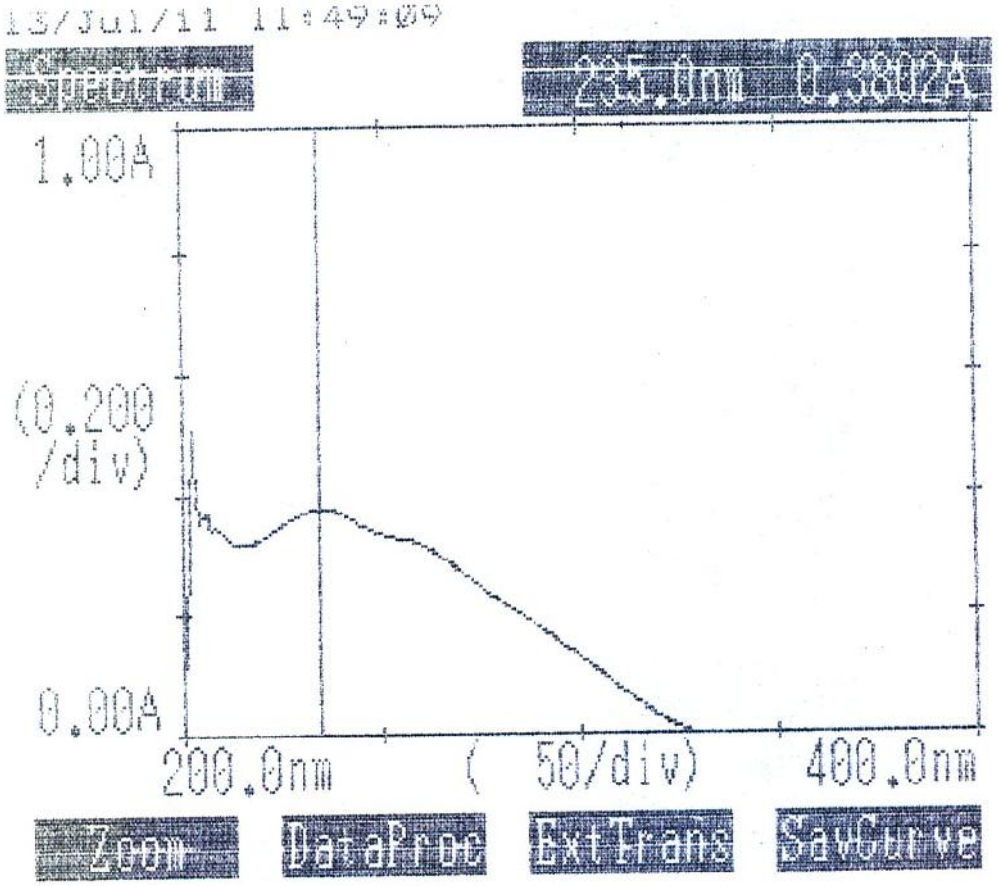


Figure-4

UV SPECTRUM OF AMBROXOL HYDROCHLORIDE IN METHANOL AT 248 nm, 308 nm (SIMULTANEOUS EQUATION METHOD)

Concentration: 10 ↘g mL^{-1}

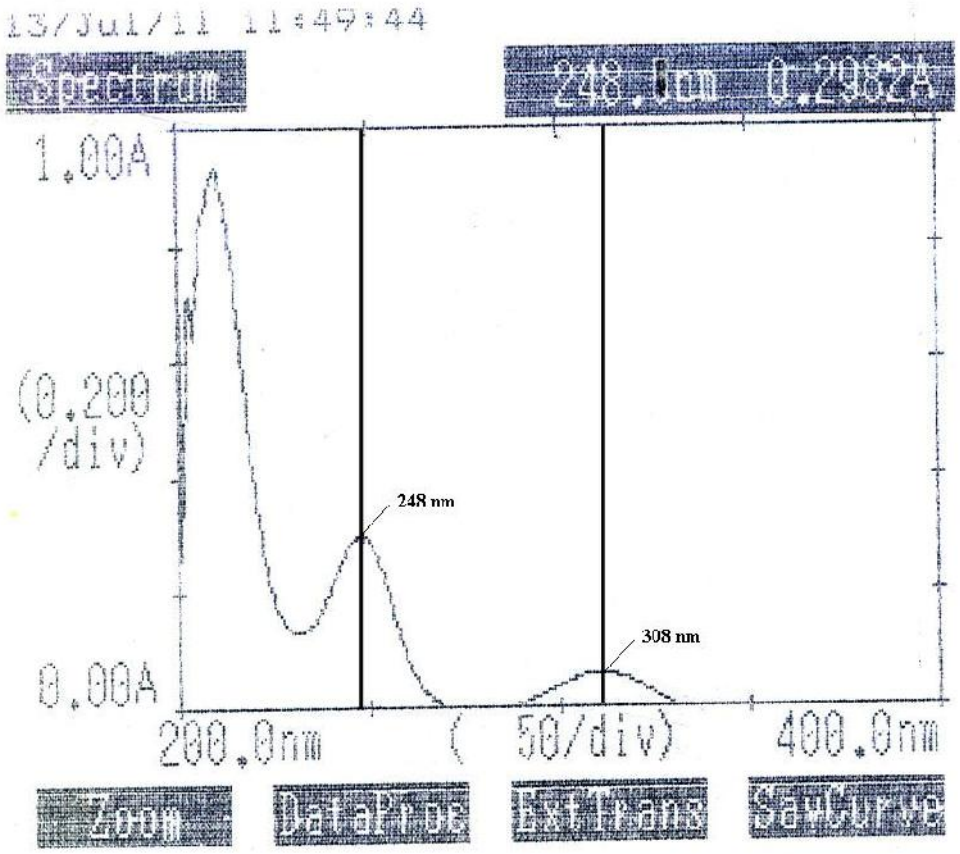


Figure-5

OVERLAID SPECTRA OF CEFPODOXIME PROXETIL AND AMBROXOL HYDROCHORIDE IN METHANOL

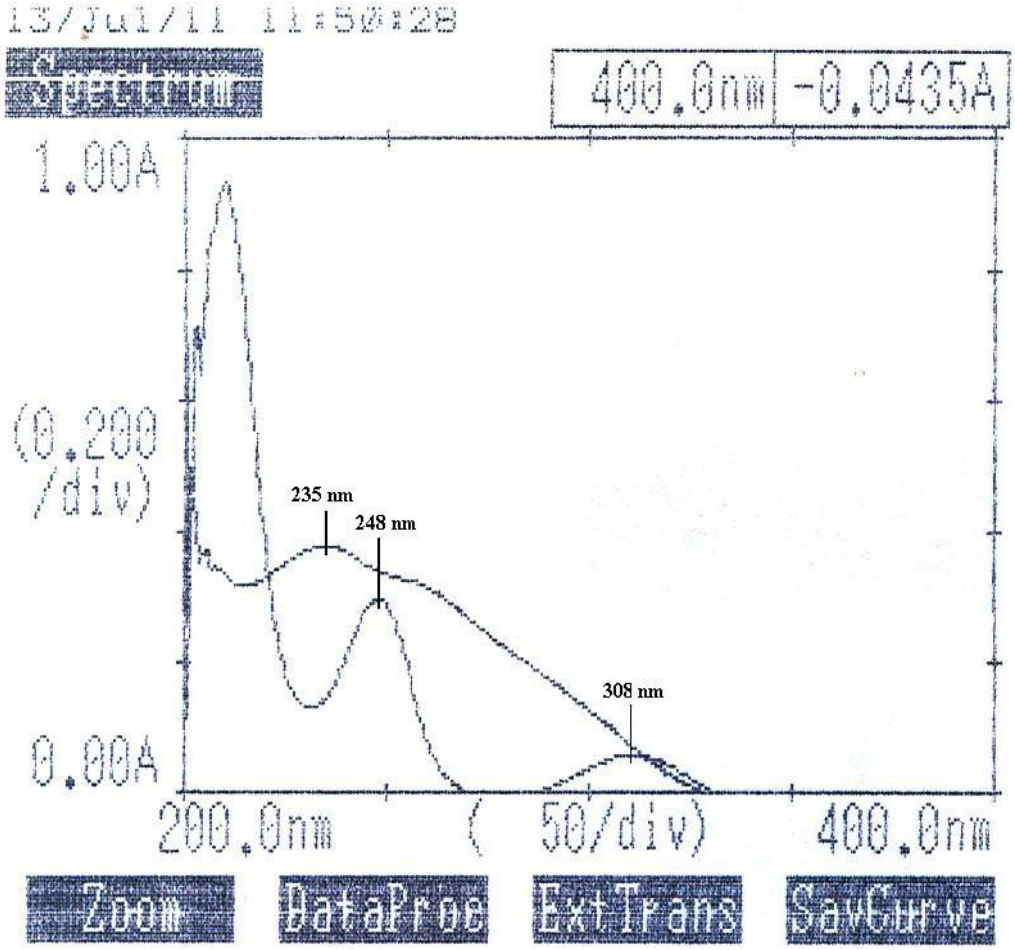


Figure-6

CALIBRATION CURVE OF CEFPODOXIME PROXETIL IN METHANOL AT 235 nm (SIMULTANEOUS EQUATION METHOD)

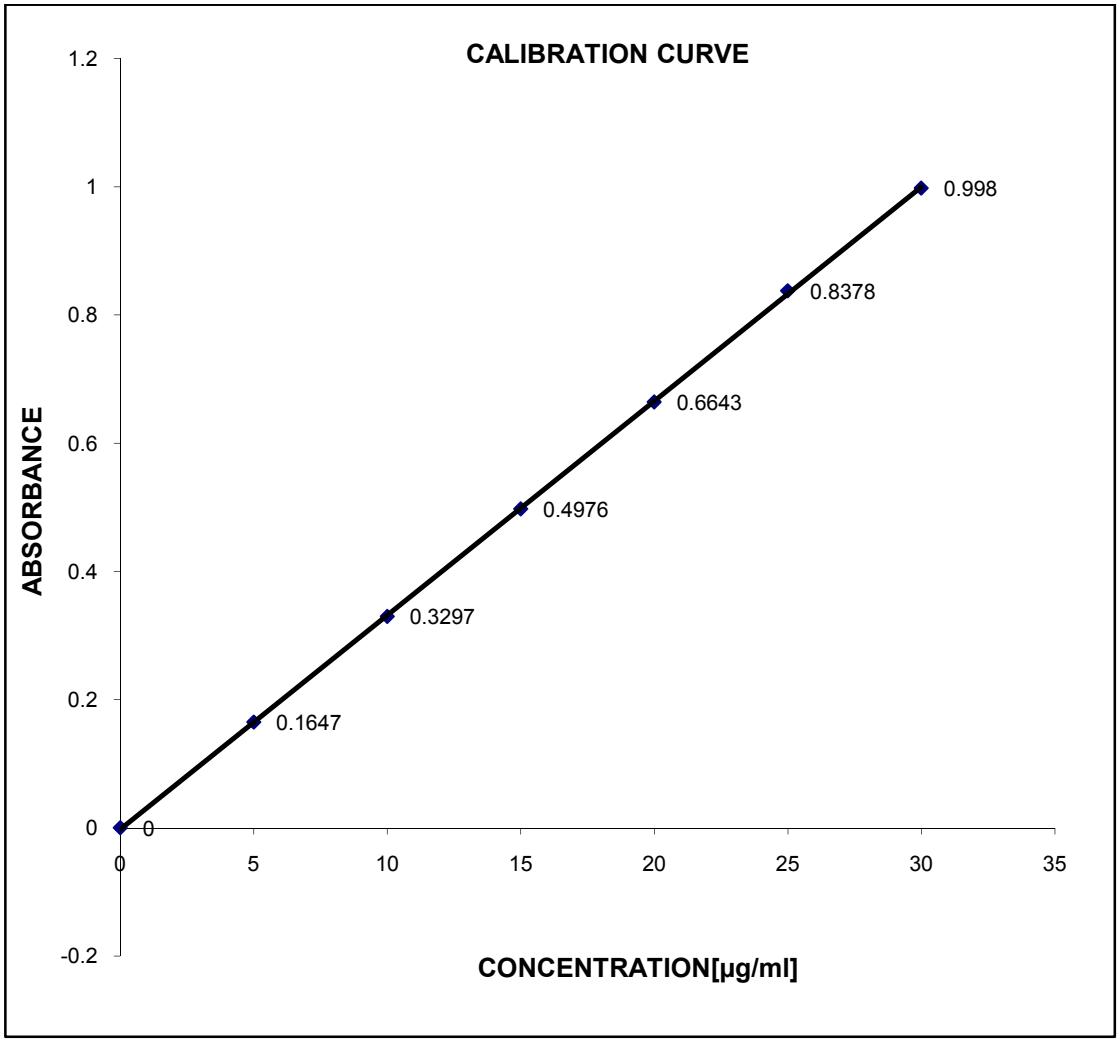


Figure-7

CALIBRATION CURVE OF CEFPODOXIME PROXETIL IN METHANOL AT 248 nm (SIMULTANEOUS EQUATION METHOD)

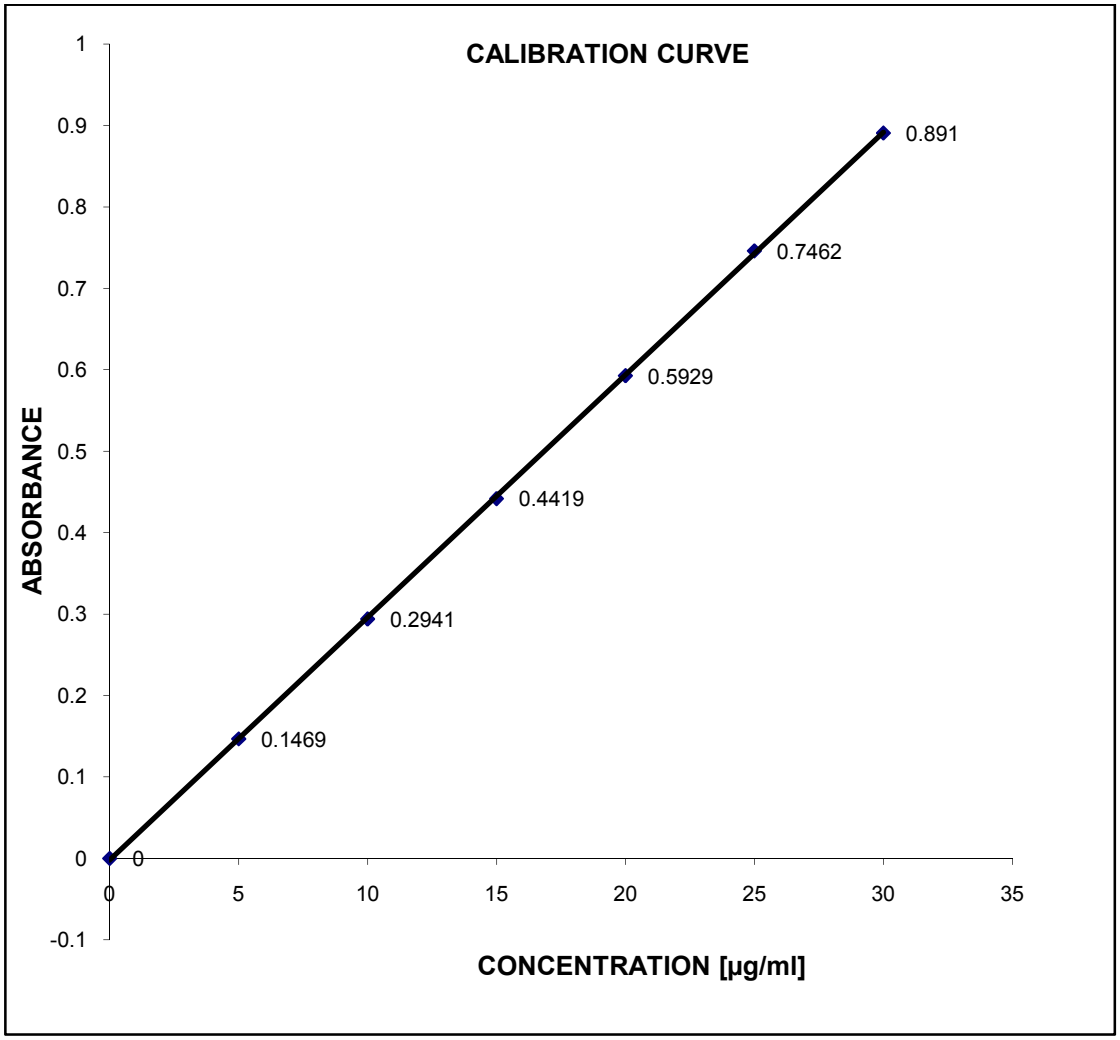


Figure-8

CALIBRATION CURVE OF CEFPODOXIME PROXETIL IN METHANOL AT 308 nm (SIMULTANEOUS EQUATION METHOD)

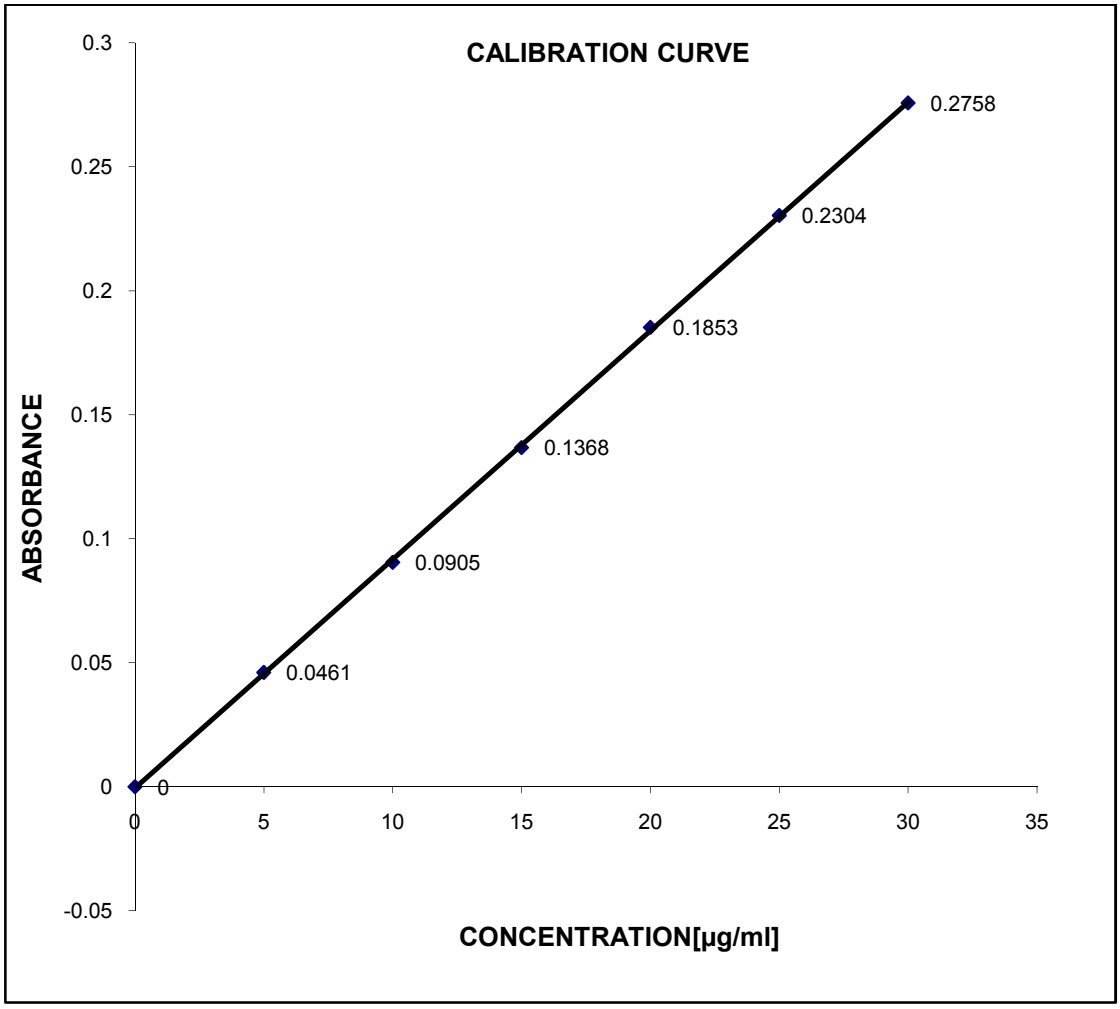


Figure-9

CALIBRATION CURVE OF AMBROXOL HYDROCHLORIDE IN METHANOL AT 235 nm

(SIMULTANEOUS EQUATION METHOD)

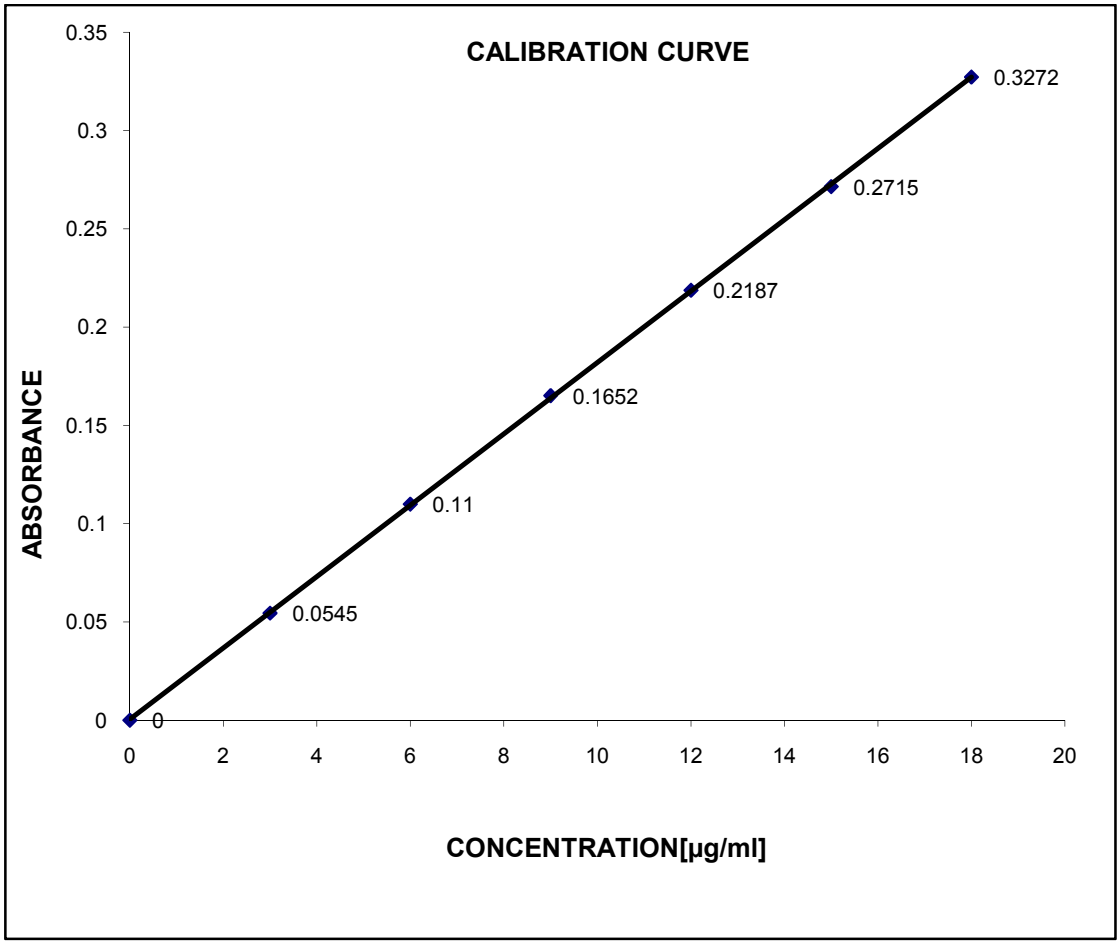


Figure-10

CALIBRATION CURVE OF AMBROXOL HYDROCHLORIDE IN METHANOL AT 248 nm

(SIMULTANEOUS EQUATION METHOD)

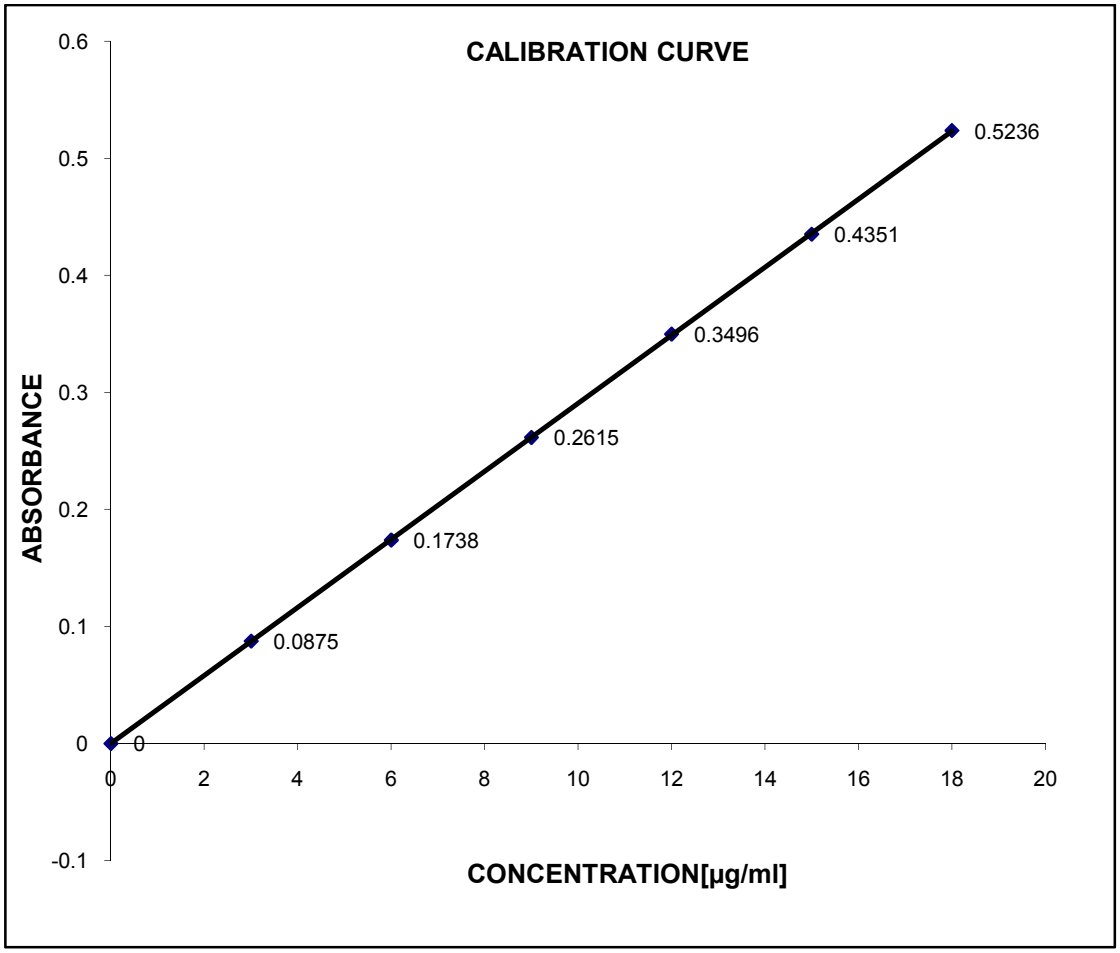


Figure-11

CALIBRATION CURVE OF AMBROXOL HYDROCHLORIDE IN METHANOL AT 308 nm

(SIMULTANEOUS EQUATION METHOD)

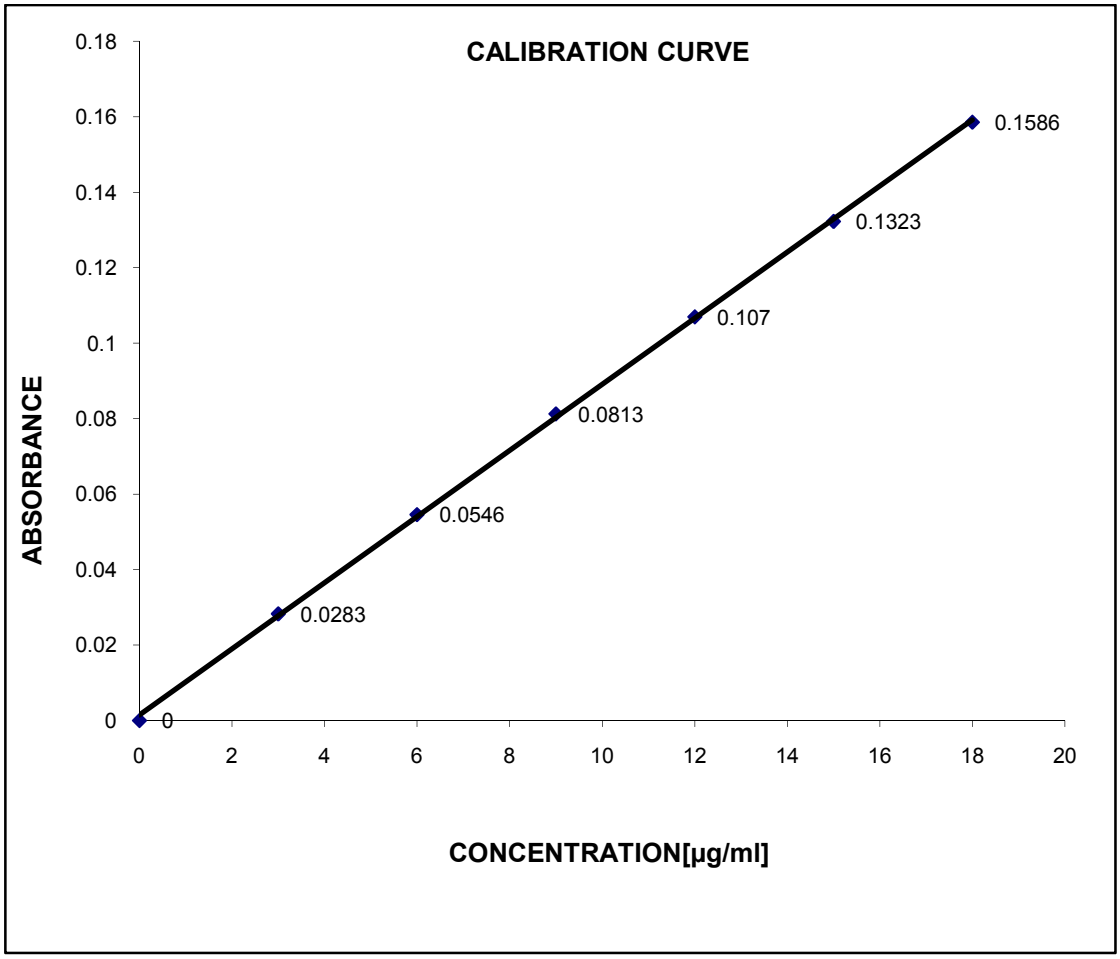


Figure-12

UV SPECTRUM OF CEFPODOXIME PROXETIL IN METHANOL

AT 229-238 nm (AREA UNDER THE CURVE METHOD)

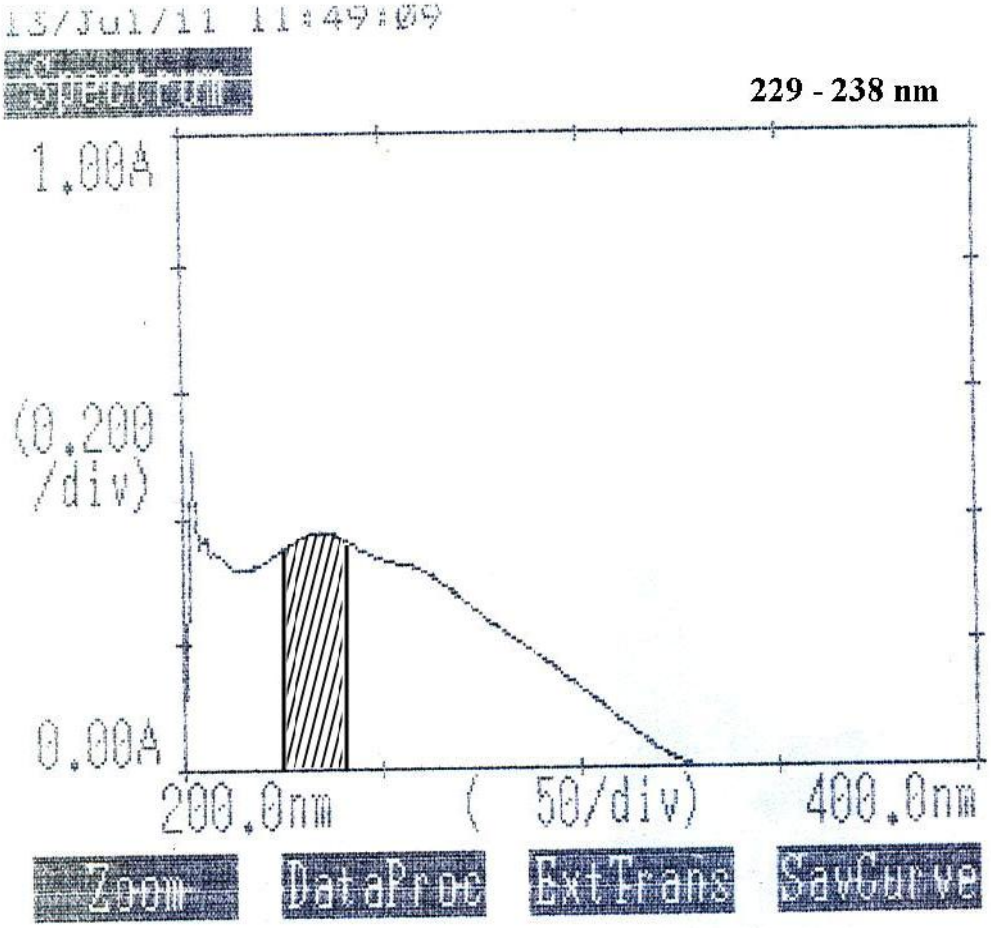


Figure-13

UV SPECTRUM OF AMBROXOL HYDROCHLORIDE IN METHANOL AT 291-316 nm (AREA UNDER THE CURVE METHOD)

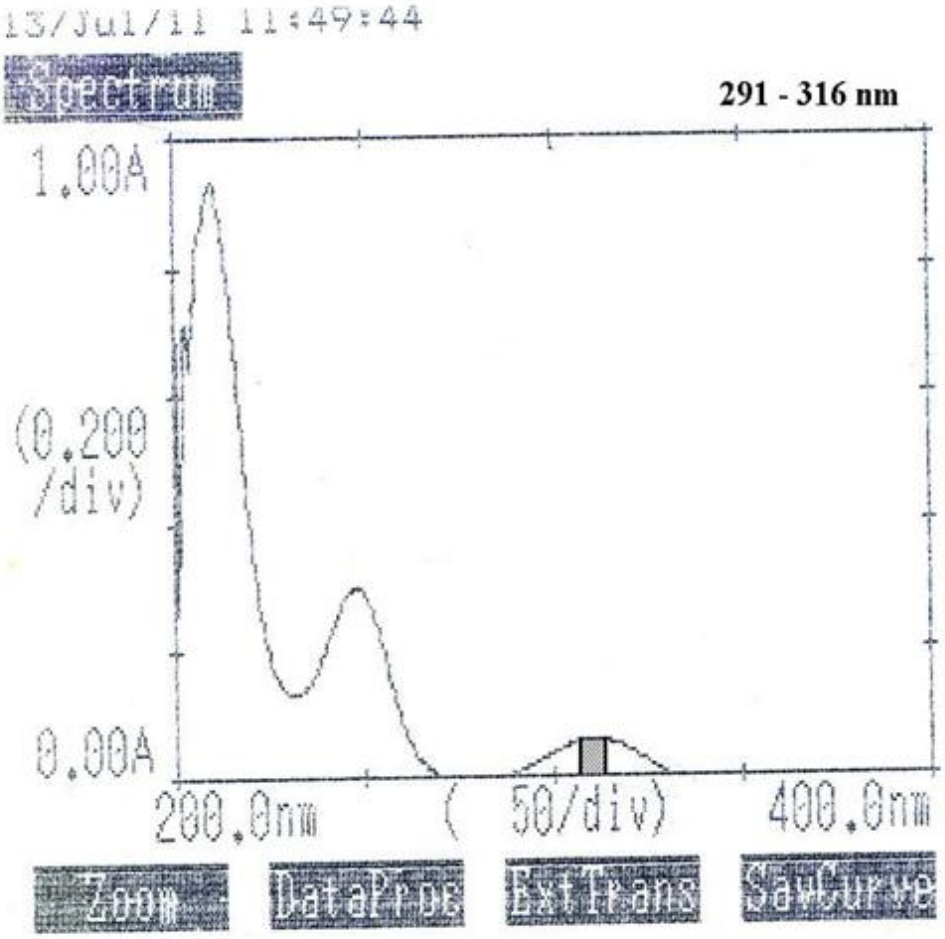


Figure-14

CALIBRATION CURVE OF CEFPODOXIME PROXETIL IN METHANOL AT 229-238 nm (AREA UNDER THE CURVE METHOD)

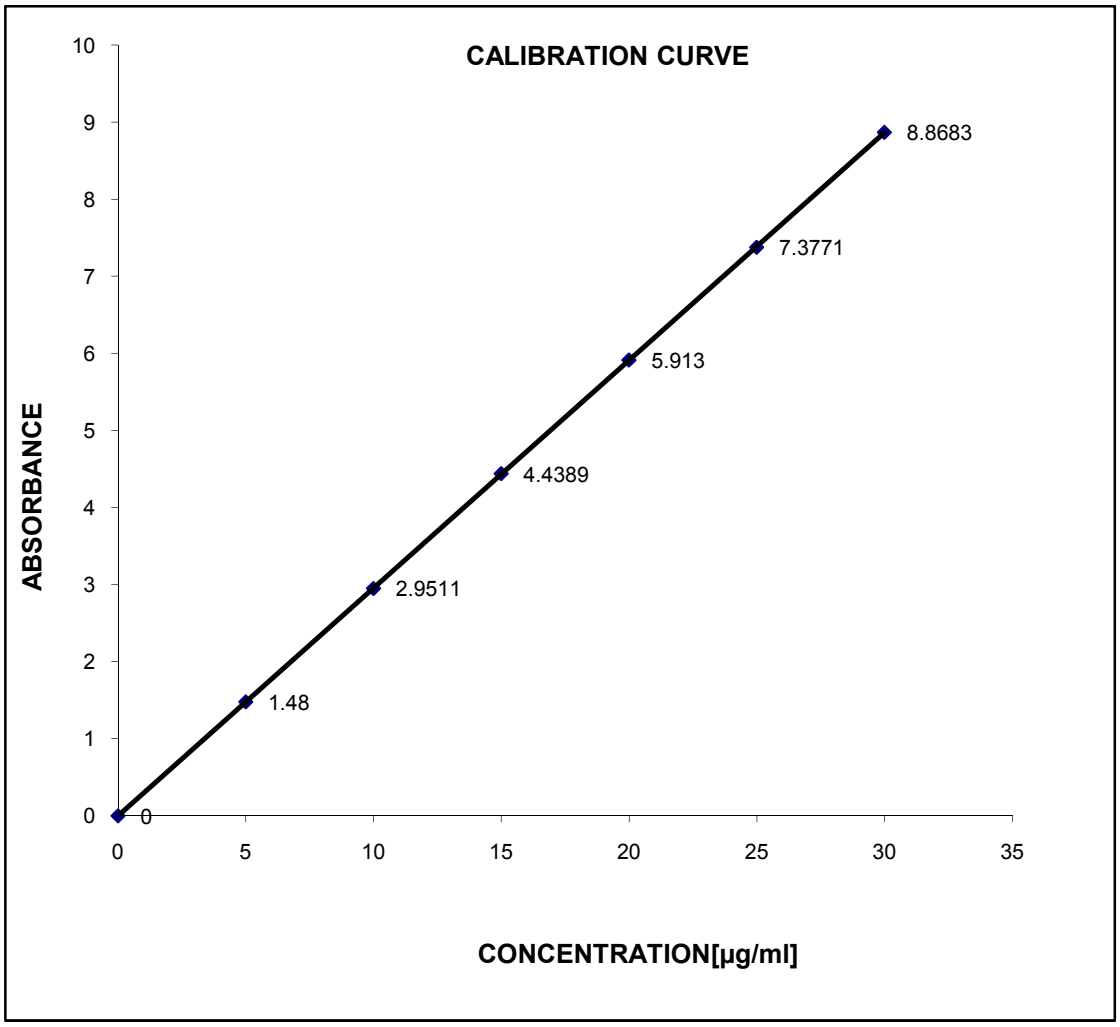


Figure-15

CALIBRATION CURVE OF CEFPODOXIME PROXETIL IN METHANOL AT 291-316 nm (AREA UNDER THE CURVE METHOD)

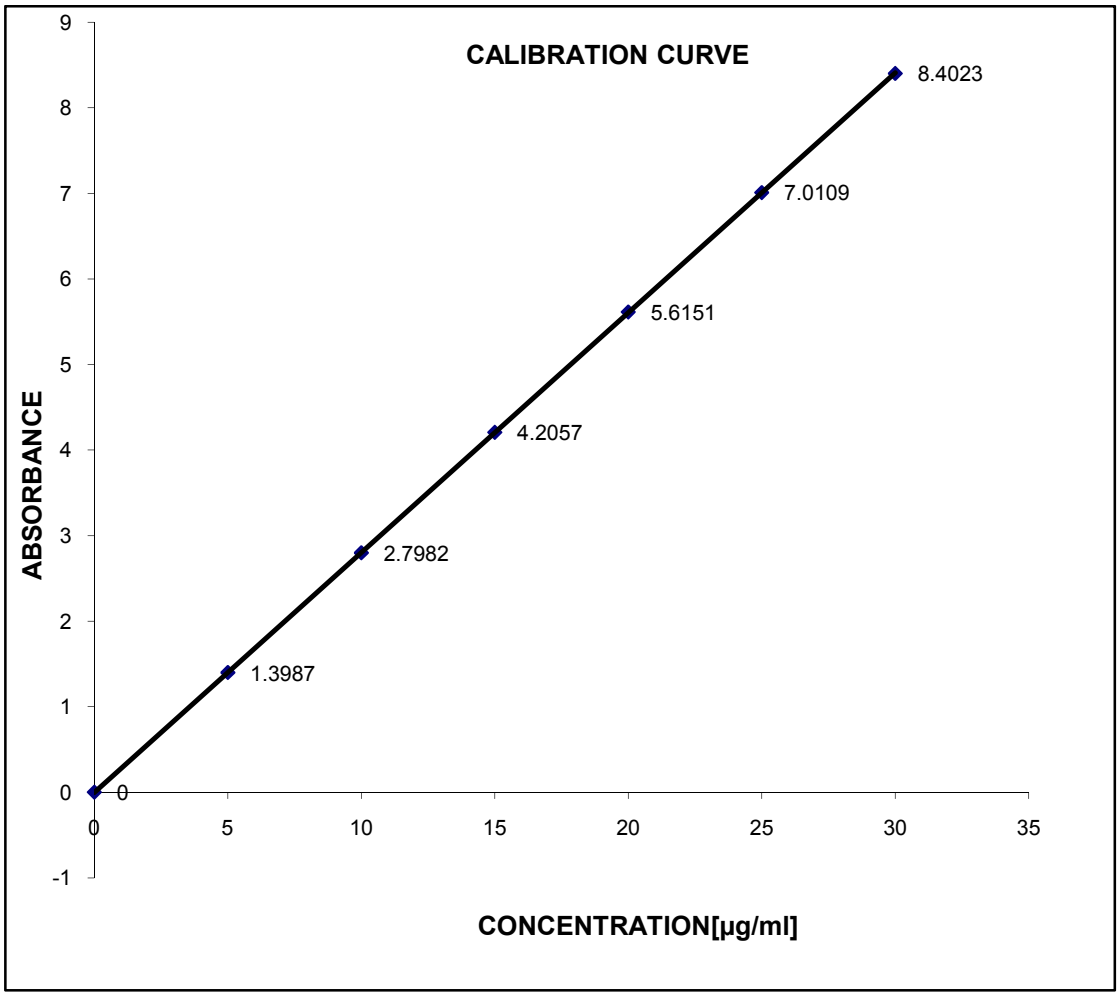


Figure-16

CALIBRATION CURVE OF AMBROXOL HYDROCHLORIDE IN METHANOL AT 229-238 nm (AREA UNDER THE CURVE METHOD)

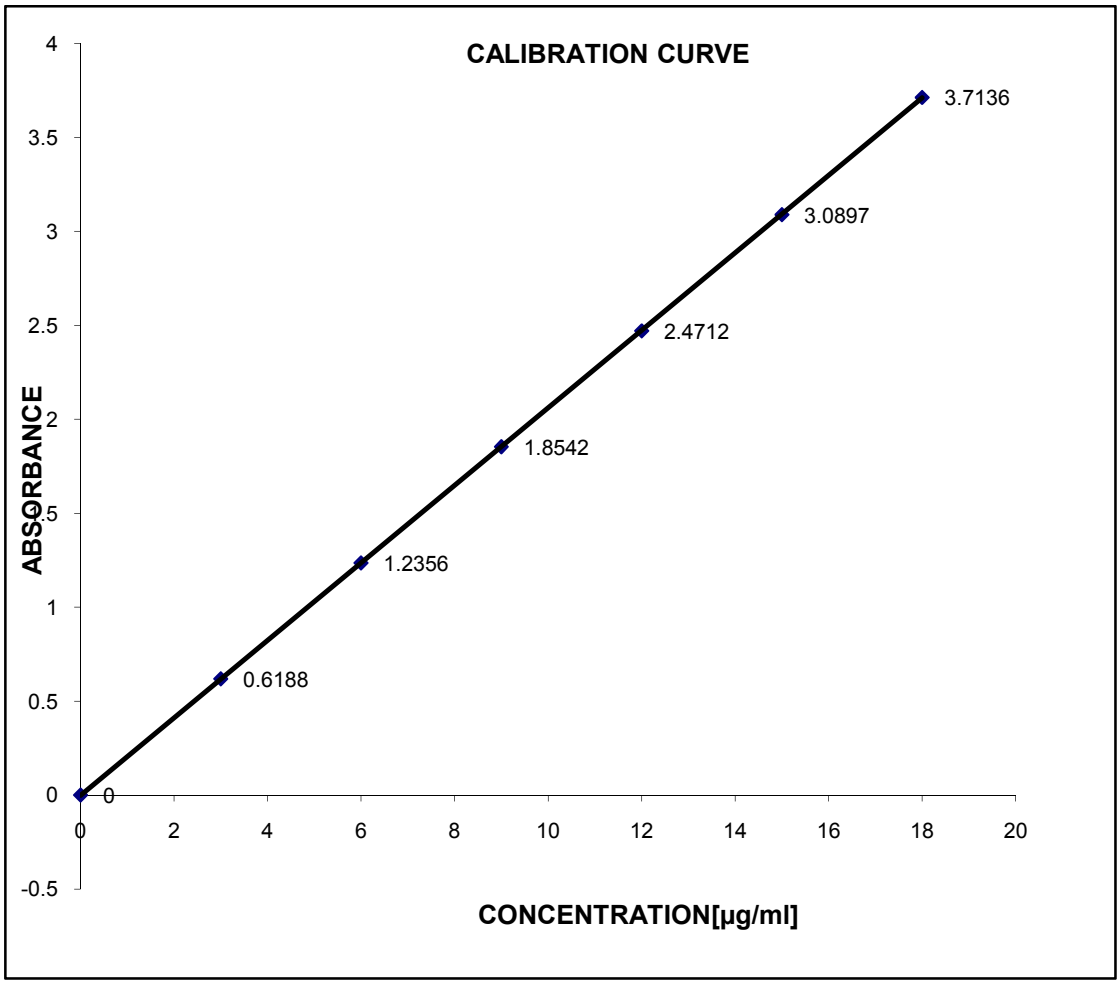


Figure-17

CALIBRATION CURVE OF AMBROXOL HYDROCHLORIDE IN METHANOL AT 291-316 nm (AREA UNDER THE CURVE METHOD)

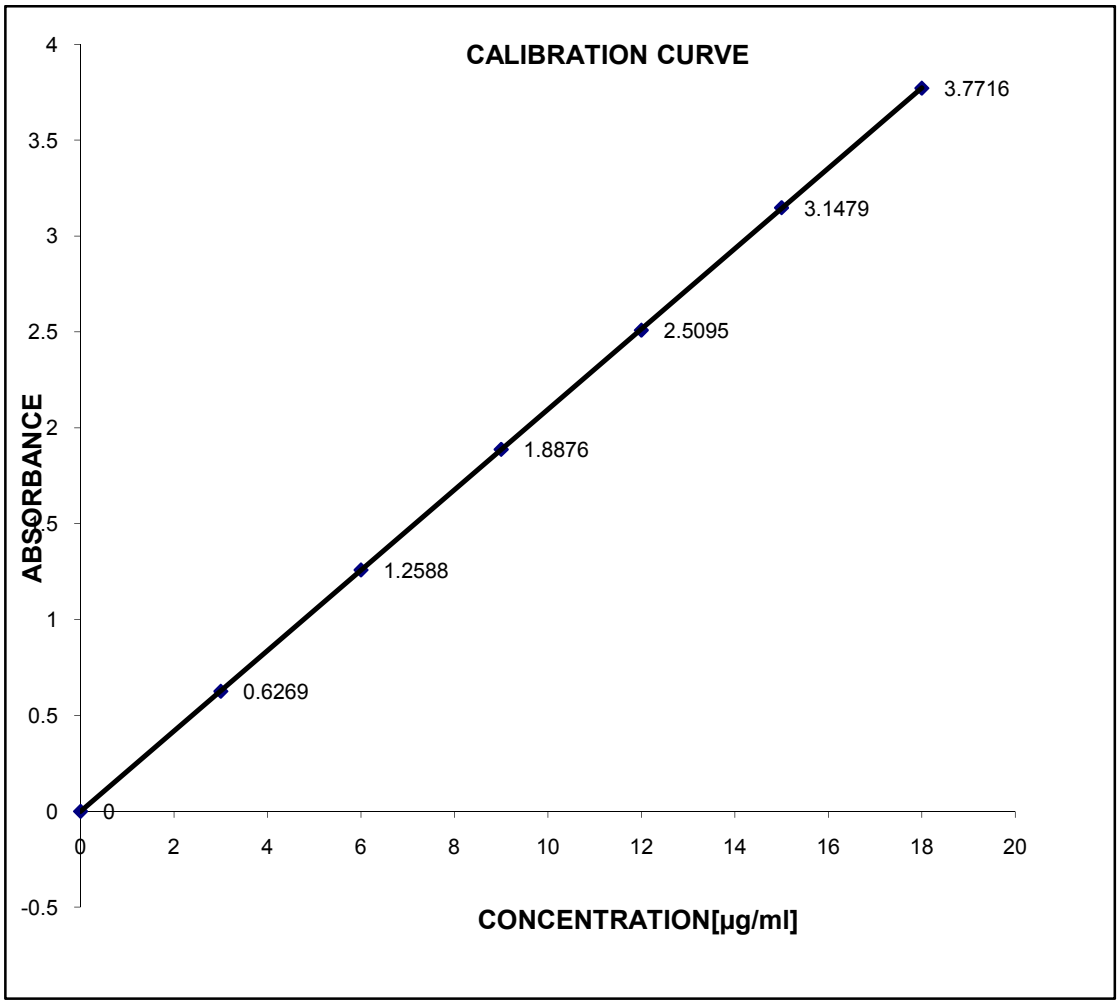


Figure-18

FIRST ORDER DERIVATIVE UV SPECTRUM OF CEFPODOXIME PROXETIL IN METHANOL

(DERIVATIVE SPECTROSCOPIC METHOD)

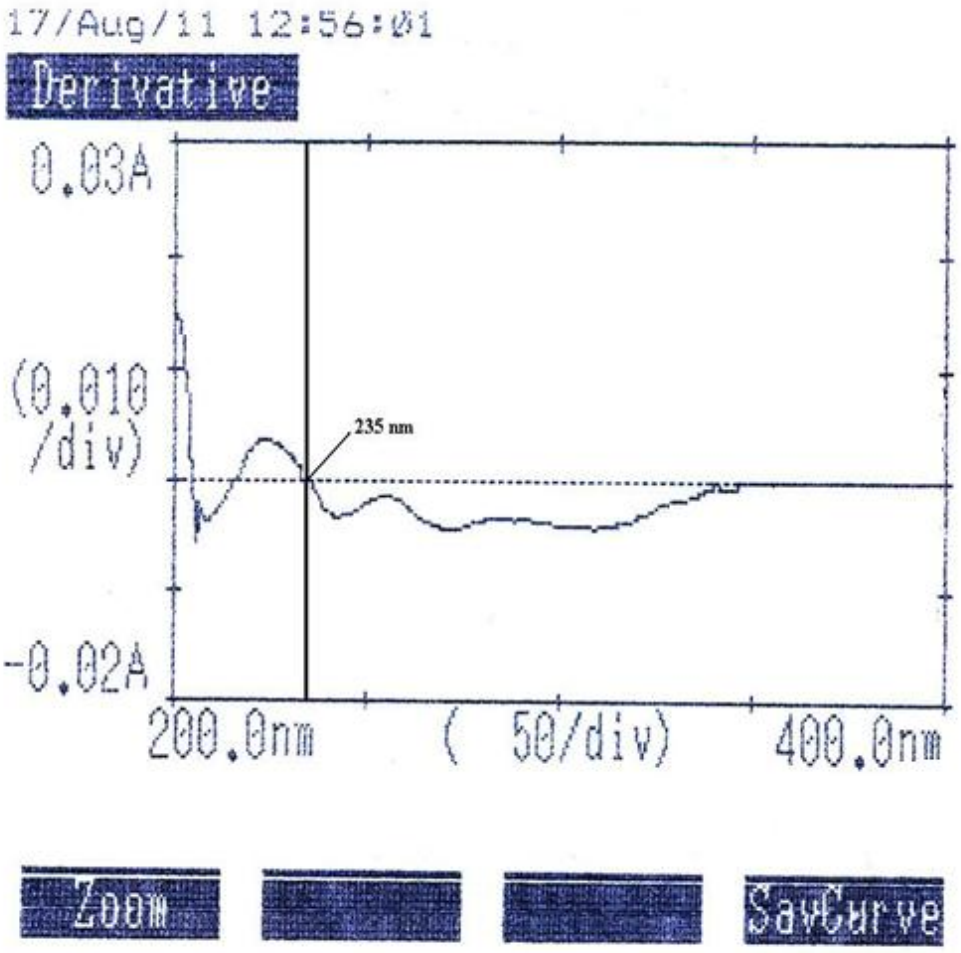


Figure-19

FIRST ORDER DERIVATIVE UV SPECTRUM OF AMBROXOL HYDROCHLORIDE IN METHANOL

(DERIVATIVE SPECTROSCOPIC METHOD)

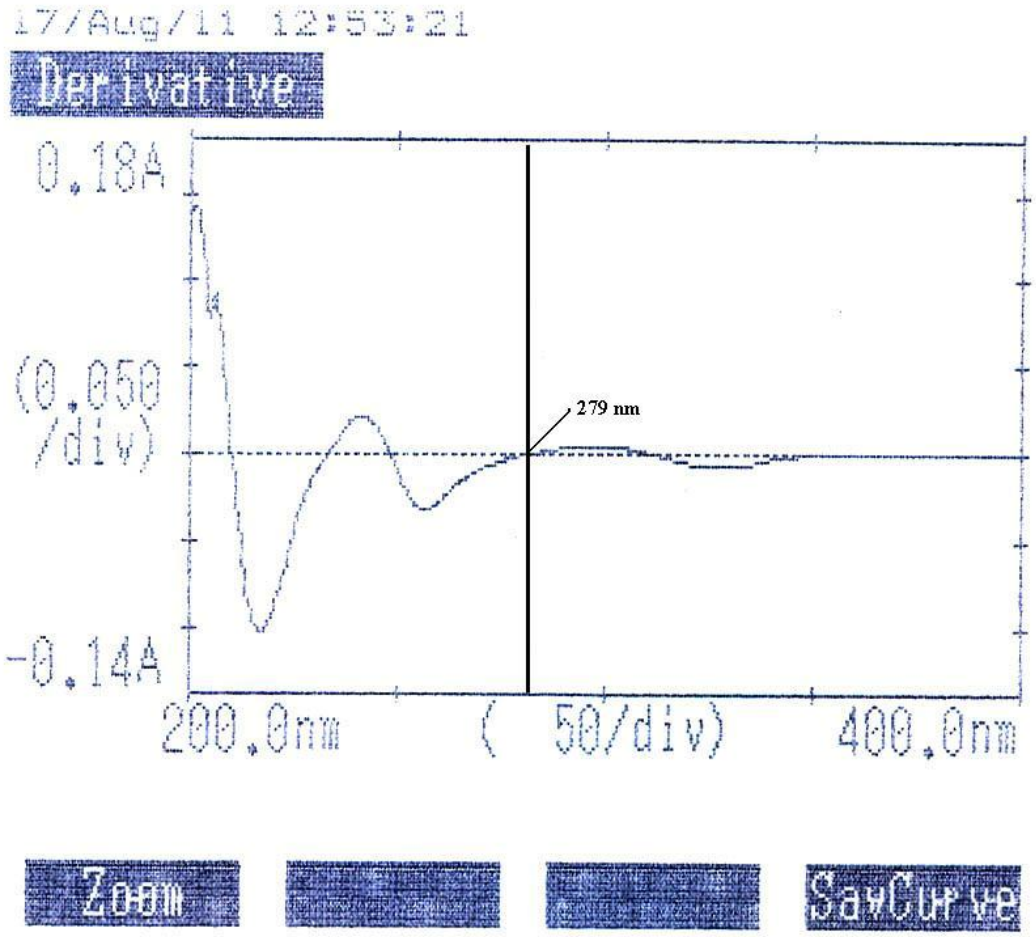


Figure-20

OVERLAID FIRST ORDER DERIVATIVE SPECTRUM OF CEFPODOXIME PROXETIL AND AMBROXOL HYDROCHORIDE IN METHANOL

(DERIVATIVE SPECTRSCOPIC METHOD)

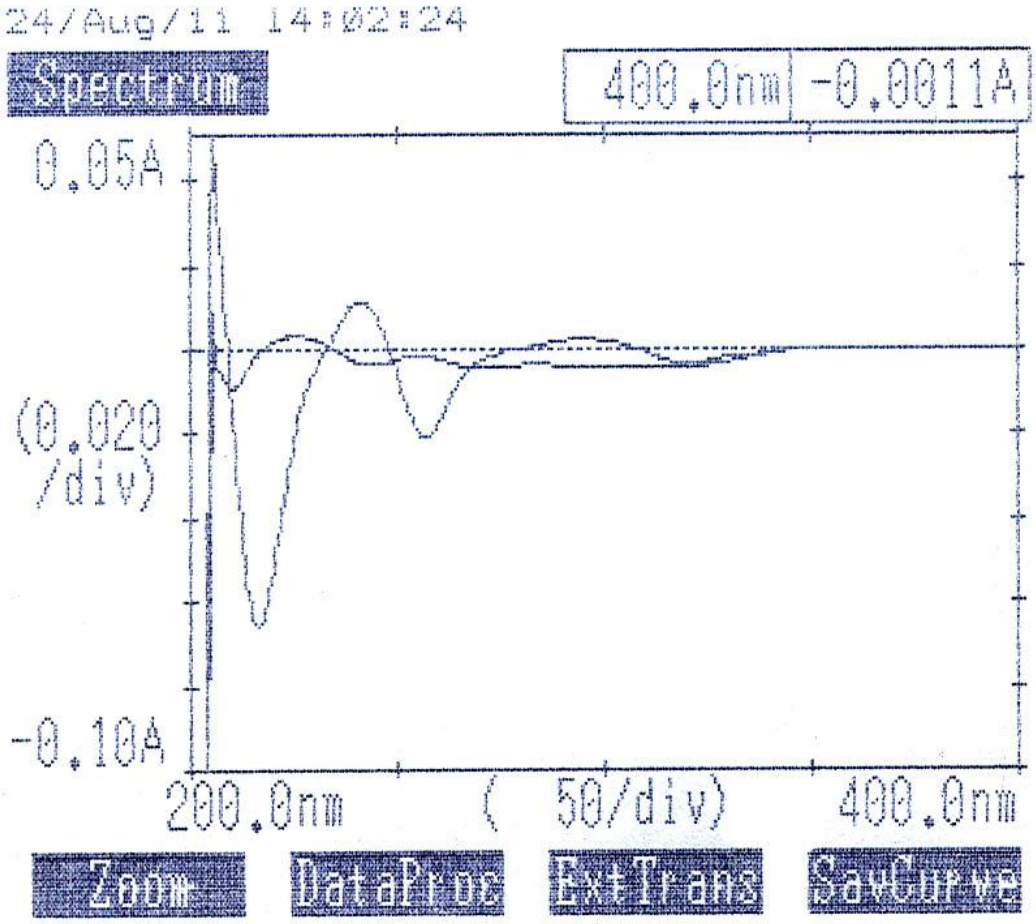


Figure-21

CALIBRATION CURVE OF CEFPODOXIME PROXETIL AT 279 nm

(DERIVATIVE SPECTROSCOPIC METHOD)

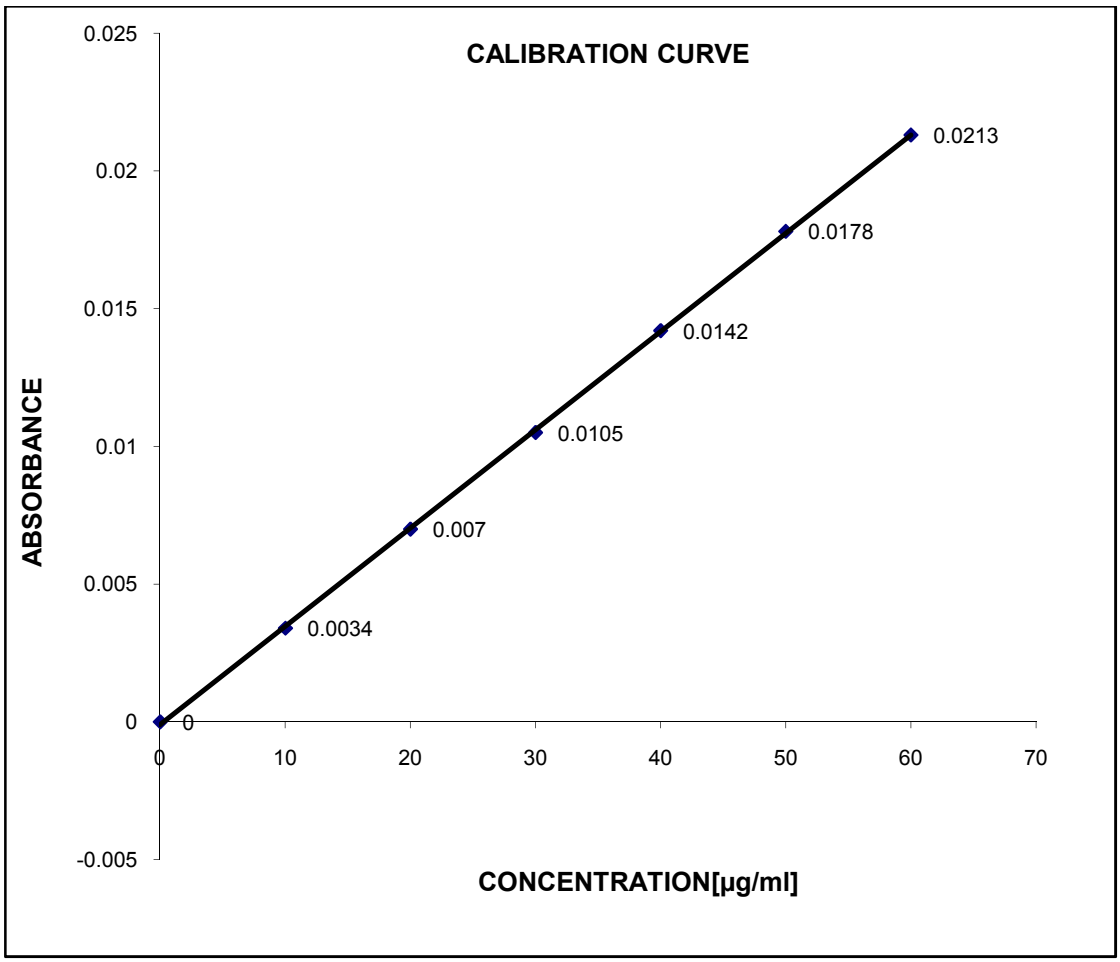


Figure-22

CALIBRATION CURVE OF AMBROXOL HYDROCHLORIDE AT 235 nm

(DERIVATIVE SPECTROSCOPIC METHOD)

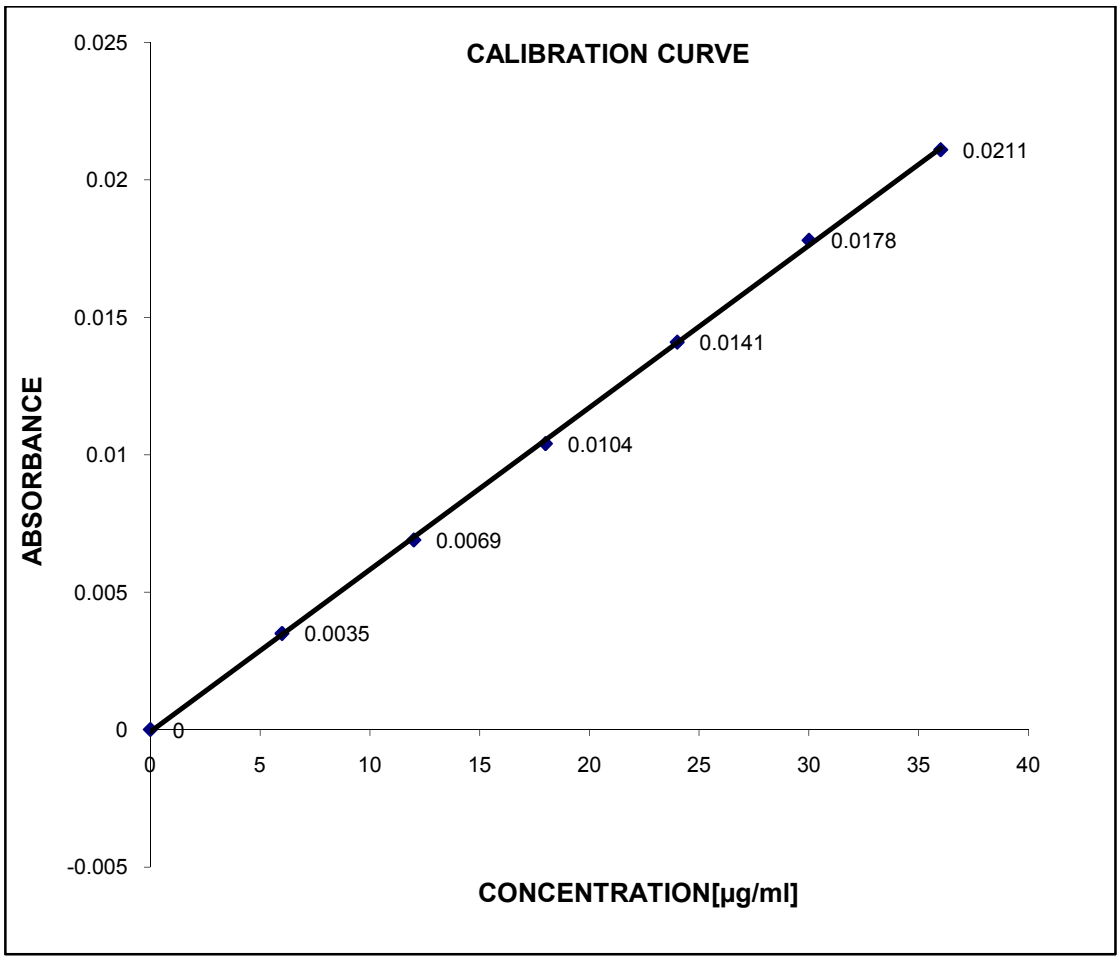
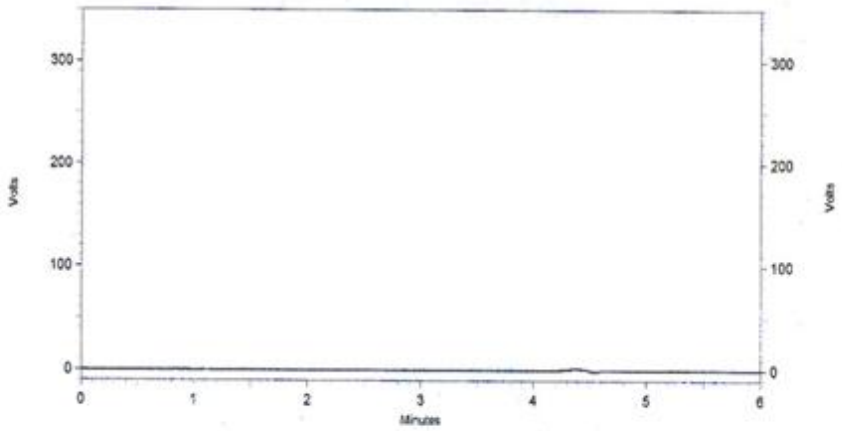


Figure-23

BLANK USING METHANOL

Area % Report

Description BLANK - METHANOL
Data File: D:\ChromQuest\PROJECTS\DATA\CEFPODOXIME + AMBROXOL\18.01.2012\0003_BLANK.dat
Method: D:\ChromQuest\Method\CEFPODOXIME + AMBROXOL.met
Acquired: 1/18/2012 8:54:04 PM
Wave Length 247 nm



Name	Retention Time	Area	Area %

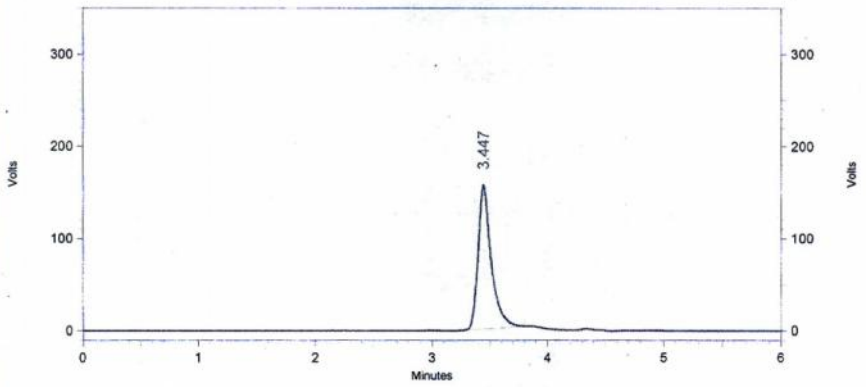
Figure 24

INITIAL SEPERATION CONDITIONS IN

ACETONITRILE: METHANOL: WATER-pH 5.0 with ORTHOPHOSPHORIC ACID (30:50:20 % v/v)

Area % Report

Description CEFPODOXIME PROXETIL
Data File: D:\ChromQuest\PROJECTS\DATA\CEFPODOXIME + AMBROXOL\18.01.2012\01_CEFPODOXIME.dat
Method: D:\ChromQuest\Method\CEFPODOXIME + AMBROXOL.met
Acquired: 1/18/2012 8:35:48 PM
Wave Length 247 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
CEFPODOXIME	3.447	1229065	100.00	168303	100.00	1.44	4952	0.00
Totals		1229065	100.00	168303	100.00			

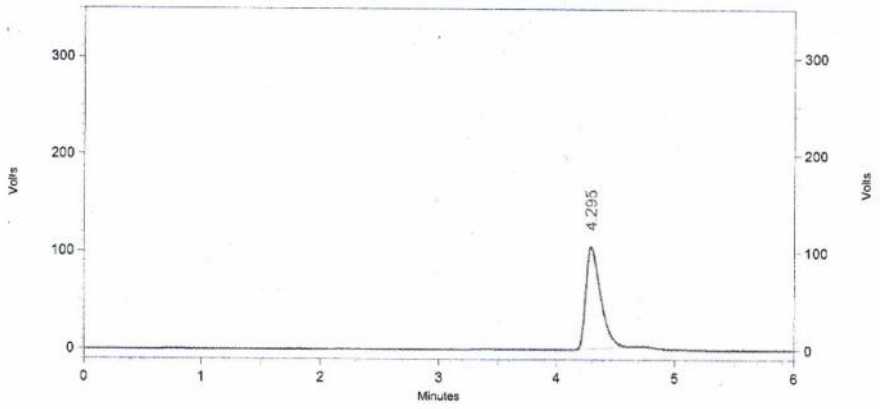
Figure 25

INITIAL SEPERATION CONDITIONS IN

ACETONITRILE: METHANOL: WATER-pH 5.0 with ORTHOPHOSPHORIC ACID (30:50:20 % v/v)

Area % Report

Description AMBROXOL HYDROCHLORIDE
Data File: D:\ChromQuest\PROJECTS\DATA\CEFPODOXIME + AMBROXOL\18.01.2012\02_AMBROXOL.dat
Method: D:\ChromQuest\Method\CEFPODOXIME + AMBROXOL.met
Acquired: 1/18/2012 8:44:55 PM
Wave Length 247 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
AMBROXOL	4.295	908886	100.00	88083	100.00	1.49	4567	0.00

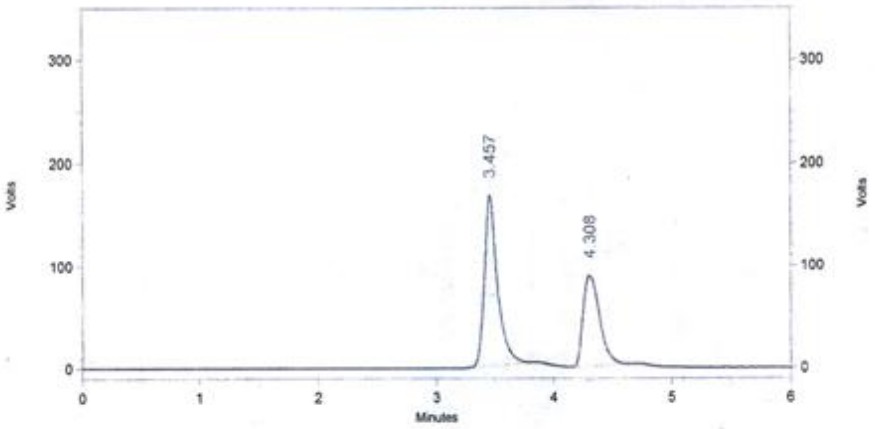
Totals								
		908886	100.00	88083	100.00			

Figure-26

OPTIMIZED CHROMATOGRAM FOR CEFPODOXIME PROXETIL AND AMBROXOL HYDROCHLORIDE

Area % Report

Description STD - Cefpodoxime (100mcg/mL) + Ambroxol (60mcg/mL)
Data File: D:\ChromQuest\PROJECTS\DATA\CEFPODOXIME + AMBROXOL\18.01.2012\002_.SS-01.dat
Method: D:\ChromQuest\Method\CEFPODOXIME + AMBROXOL.met
Acquired: 1/18/2012 4:47:32 PM
Wave Length 247 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
CEFPODOXIME	3.457	1289121	59.90	166303	65.37	1.44	4962	0.00
AMBROXOL	4.308	863078	40.10	88083	34.63	1.49	4637	3.79
Totals		2152199	100.00	254386	100.00			

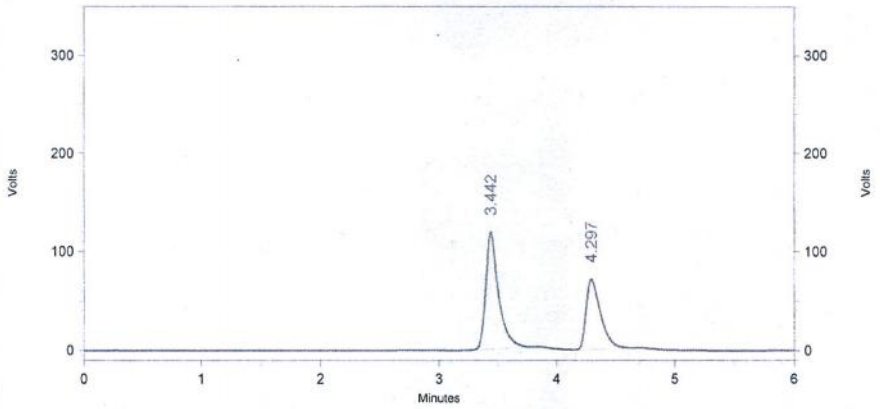
Figure-27

LINEARITY CHROMATOGRAM OF CEFPODOXIME PROXETIL AND AMBROXOL HYDROCHLORIDE

(70, 42 µg/ ml)

Area % Report

Description STD 70% CONCENTRATION- Cefpodoxime (70mcg/mL) + Ambroxol (42mcg/mL)
Data File: D:\ChromQuest\PROJECTS\DATA\CEFPODOXIME + AMBROXOL\18.01.2012\007_LINEARITY-70%.dat
Method: D:\ChromQuest\Method\CEFPODOXIME + AMBROXOL.met
Acquired: 1/18/2012 5:33:12 PM
Wave Length 247 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
CEFPODOXIME	3.442	886875	59.63	118293	62.65	1.55	5316	0.00
AMBROXOL	4.297	600390	40.37	70510	37.35	1.66	5812	4.13
Totals		1487265	100.00	188803	100.00			

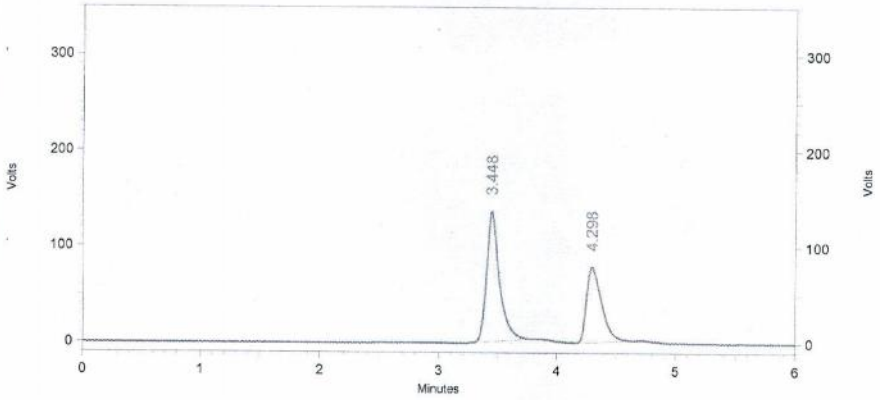
Figure-28

LINEARITY CHROMATOGRAM OF CEFPODOXIME PROXETIL AND AMBROXOL HYDROCHLORIDE

(80, 48 µg/ ml)

Area % Report

Description STD 80% CONCENTRATION- Cefpodoxime (80mcg/mL) + Ambroxol (48mcg/mL)
Data File: D:\ChromQuest\PROJECTS\DATA\CEFPODOXIME + AMBROXOL\18.01.2012\008_LINEARITY-80%.dat
Method: D:\ChromQuest\Method\CEFPODOXIME + AMBROXOL.met
Acquired: 1/18/2012 5:42:21 PM
Wave Length 247 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
CEFPODOXIME	3.448	1022577	59.71	135350	63.47	1.50	5264	0.00
AMBROXOL	4.298	689933	40.29	77902	36.53	1.67	5210	3.97
Totals		1712510	100.00	213252	100.00			

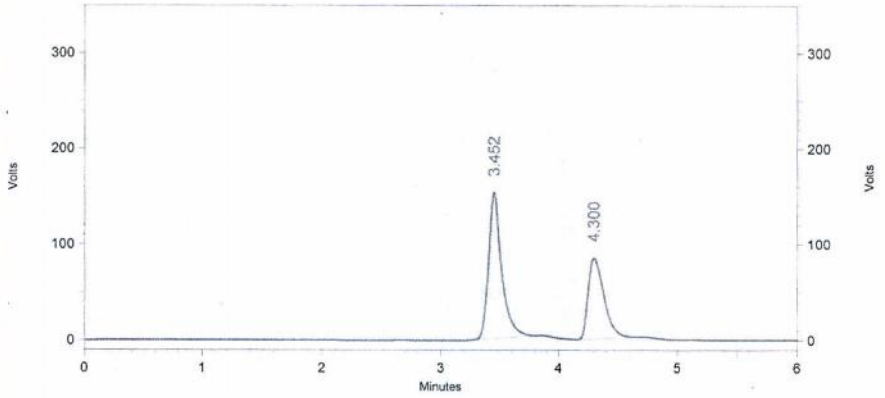
Figure-29

LINEARITY CHROMATOGRAM OF CEFPODOXIME PROXETIL AND AMBROXOL HYDROCHLORIDE

(90, 54 µg/ ml)

Area % Report

Description STD 90% CONCENTRATION- Cefpodoxime (90mcg/mL) + Ambroxol (54mcg/mL)
Data File: D:\ChromQuest\PROJECTS\DATA\CEFPODOXIME + AMBROXOL\18.01.2012\009_LINEARITY-90%.dat
Method: D:\ChromQuest\Method\CEFPODOXIME + AMBROXOL.met
Acquired: 1/18/2012 5:51:29 PM
Wave Length 247 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
CEFPODOXIME	3.452	1162165	59.86	151997	64.41	1.46	5112	0.00
AMBROXOL	4.300	779200	40.14	84000	35.59	1.63	4841	3.85
Totals		1941365	100.00	235997	100.00			

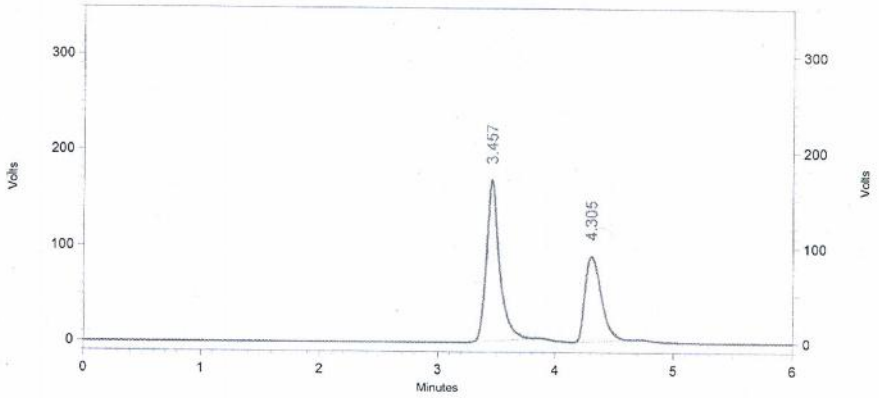
Figure-30

LINEARITY CHROMATOGRAM OF CEFPODOXIME PROXETIL AND AMBROXOL HYDROCHLORIDE

(100, 60 µg/ ml)

Area % Report

Description STD 100% CONCENTRATION- Cefpodoxime (100mcg/mL) + Ambroxol (60mcg/mL)
Data File: D:\ChromQuest\PROJECTS\DATA\CEFPODOXIME + AMBROXOL\18.01.2012\010_LINEARITY-100%.dat
Method: D:\ChromQuest\Method\CEFPODOXIME + AMBROXOL.met
Acquired: 1/18/2012 6:00:36 PM
Wave Length 247 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
CEFPODOXIME	3.457	1304227	60.09	168146	65.27	1.45	4972	0.00
AMBROXOL	4.305	866229	39.91	89452	34.73	1.58	4680	3.79
Totals		2170456	100.00	257598	100.00			

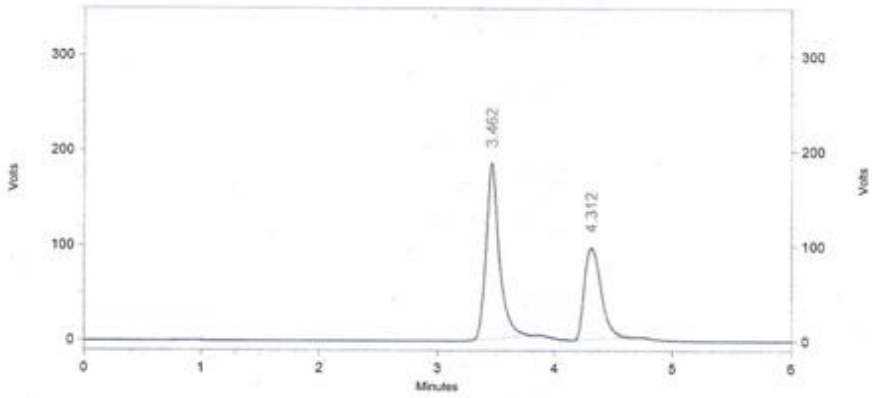
Figure-31

LINEARITY CHROMATOGRAM OF CEFPODOXIME PROXETIL AND AMBROXOL HYDROCHLORIDE

(110, 66 µg/ ml)

Area % Report

Description STD 110% CONCENTRATION- Cefpodoxime (110mcg/mL) + Ambroxol (66mcg/mL)
Data File: D:\ChromQuest\PROJECTS\DATA\CEFPODOXIME + AMBROXOL\18.01.2012\011_LINEARITY-110%.dat
Method: D:\ChromQuest\Method\CEFPODOXIME + AMBROXOL.met
Acquired: 1/18/2012 6:09:45 PM
Wave Length 247 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
CEFPODOXIME	3.462	1448450	60.20	184484	65.73	1.41	4860	0.00
AMBROXOL	4.312	957608	39.80	96167	34.27	1.50	4579	3.75
Totals		2406058	100.00	280651	100.00			

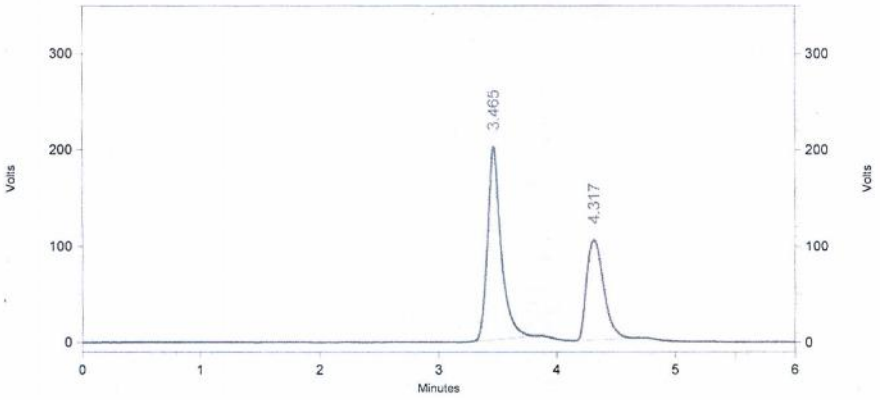
Figure-32

LINEARITY CHROMATOGRAM OF CEFPODOXIME PROXETIL AND AMBROXOL HYDROCHLORIDE

(120, 72 µg/ ml)

Area % Report

Description STD 120% CONCENTRATION- Cefpodoxime (120mcg/mL) + Ambroxol (72mcg/mL)
Data File: D:\ChromQuest\PROJECTS\DATA\CEFPODOXIME + AMBROXOL\18.01.2012\012_LINEARITY-120%.dat
Method: D:\ChromQuest\Method\CEFPODOXIME + AMBROXOL.met
Acquired: 1/18/2012 6:18:52 PM
Wave Length 247 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
CEFPODOXIME	3.465	1591153	60.40	200326	65.87	1.37	4731	0.00
AMBROXOL	4.317	1043159	39.60	103813	34.13	1.41	4445	3.70
Totals		2634312	100.00	304139	100.00			

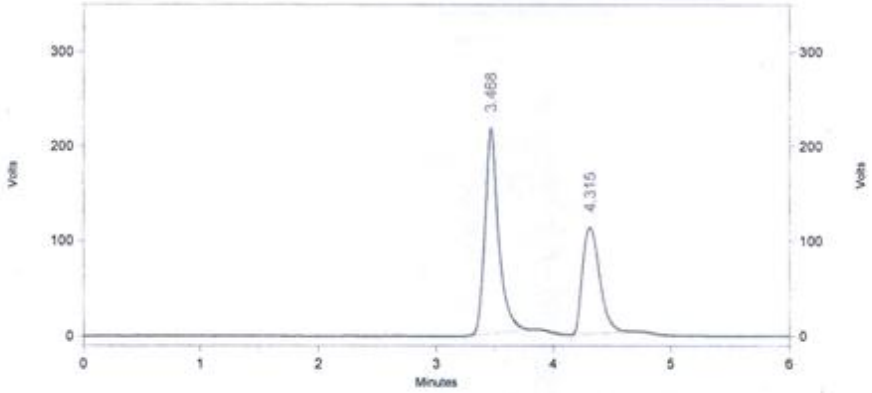
Figure-33

LINEARITY CHROMATOGRAM OF CEFPODOXIME PROXETIL AND AMBROXOL HYDROCHLORIDE

(130, 78 µg/ ml)

Area % Report

Description STD 130% CONCENTRATION- Cefpodoxime (130mcg/mL) + Ambroxol (78mcg/mL)
Data File: D:\ChromQuest\PROJECTS\DATA\CEFPODOXIME + AMBROXOL\18.01.2012\013_LINEARITY-130%.dat
Method: D:\ChromQuest\Method\CEFPODOXIME + AMBROXOL.met
Acquired: 1/18/2012 6:28:00 PM
Wave Length 247 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
CEFPODOXIME	3.468	1747353	60.54	216360	66.04	1.35	4550	0.00
AMBROXOL	4.315	1138688	39.46	111250	33.96	1.40	4219	3.59
Totals		2886041	100.00	327610	100.00			

Figure-34

CALIBRATION CURVE OF CEFPODOXIME PROXETIL BY RP-HPLC

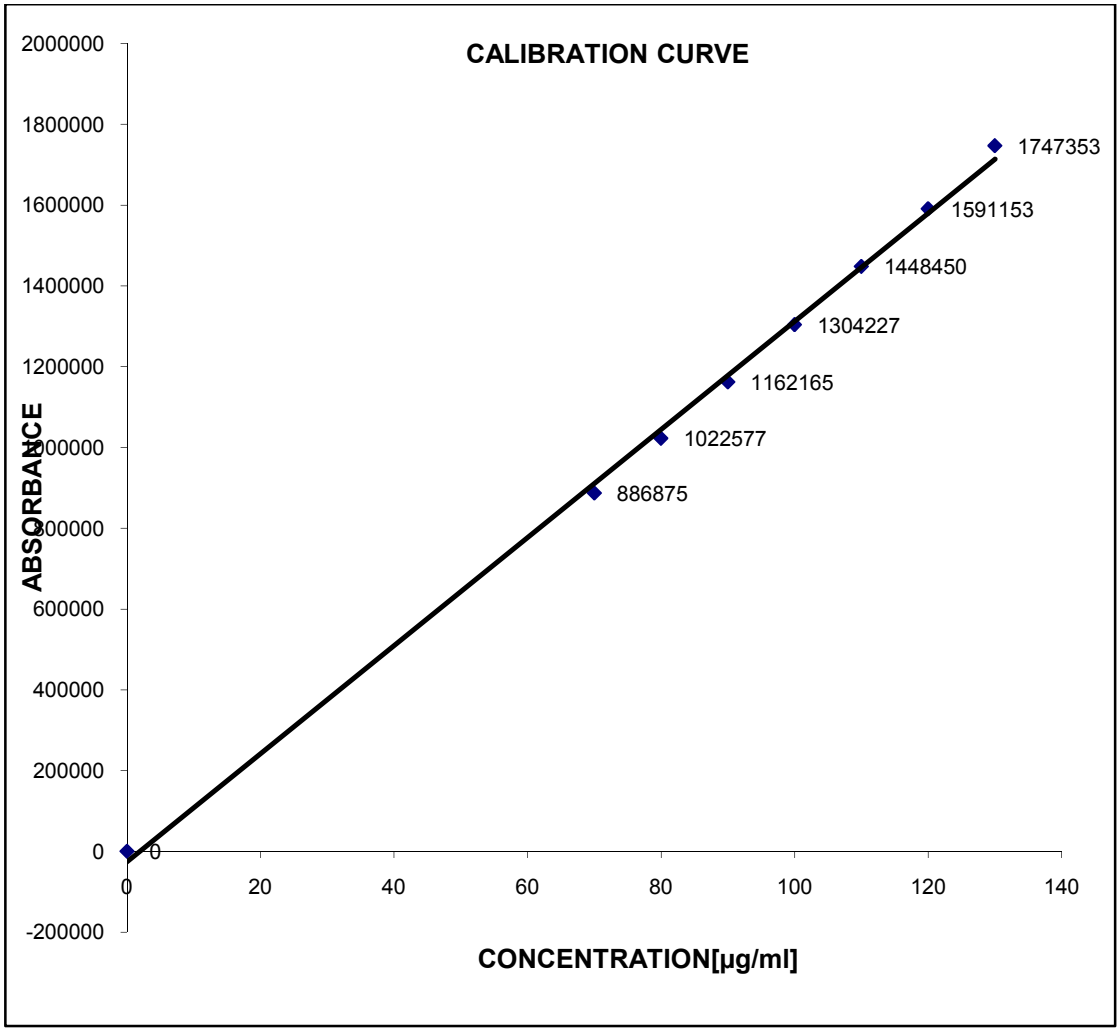


Figure-35

CALIBRATION CURVE OF AMBROXOL HYDROCHLORIDE BY RP-HPLC

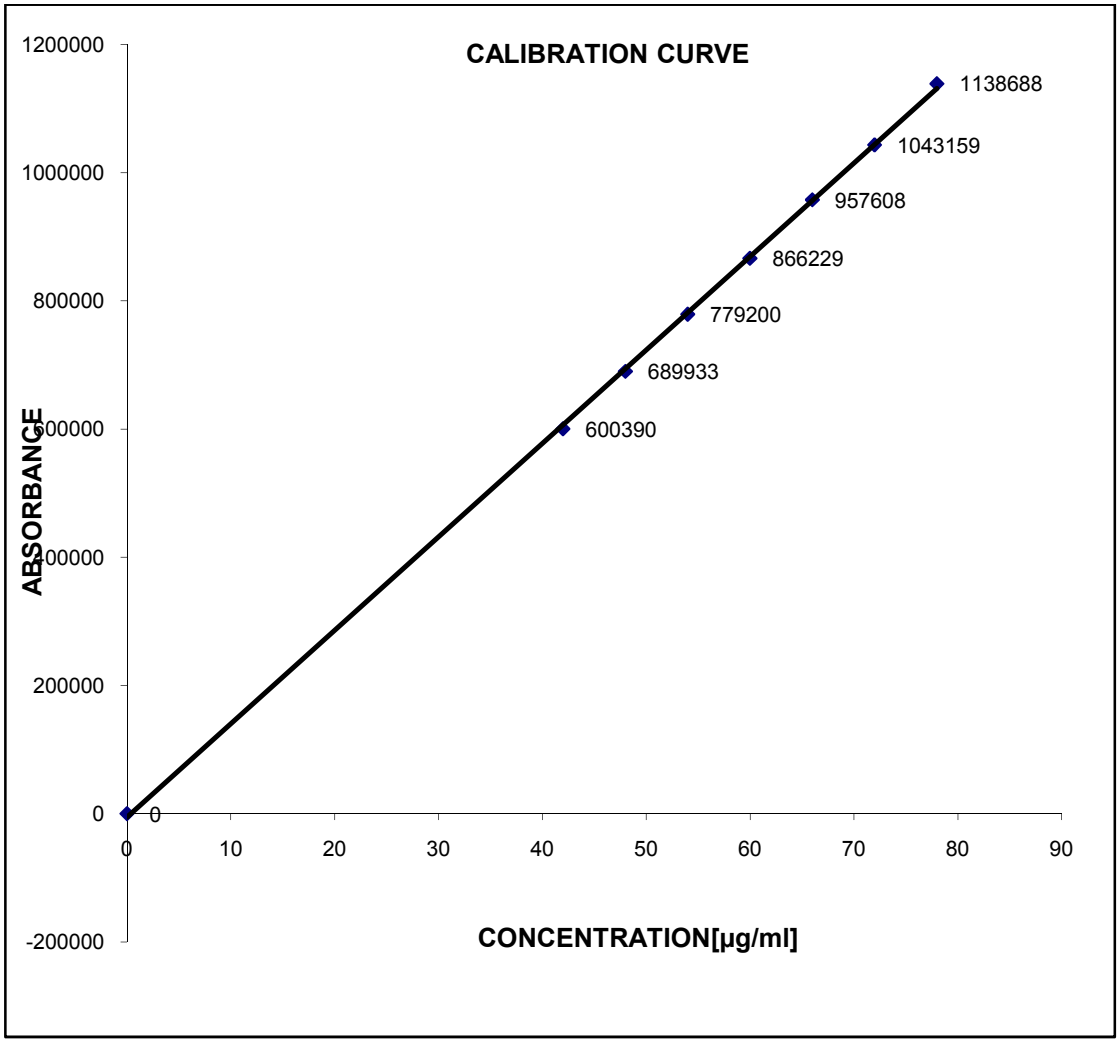
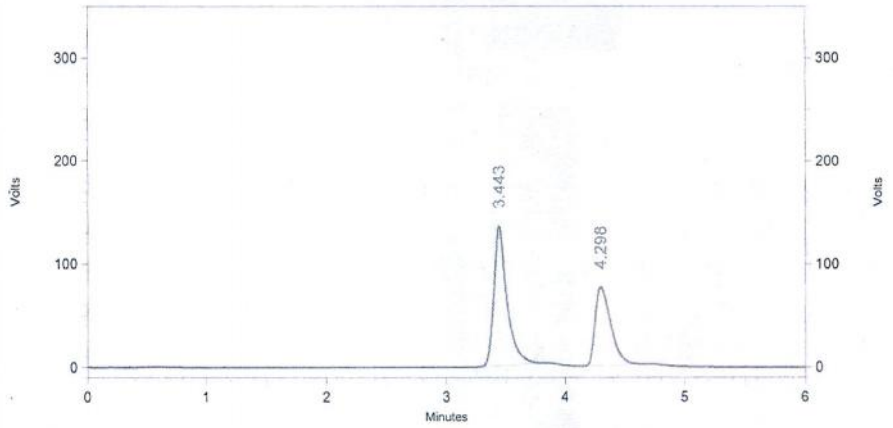


Figure-36

CHROMATOGRAM FOR ANALYSIS OF FOMULATION FINECEF-AM TAB FOR LOW LEVEL DILUTONS REPEATABILITY - 1

Area % Report

Description SPL- 01_FORMULATION - LOW LEVEL CONCENTRATION - 80%
Data File: D:\ChromQuest\PROJECTS\DATA\CEFPODOXIME + AMBROXOL\18.01.2012\014_PA_L-01.dat
Method: D:\ChromQuest\Method\CEFPODOXIME + AMBROXOL.met
Acquired: 1/18/2012 6:37:08 PM
Wave Length 247 nm



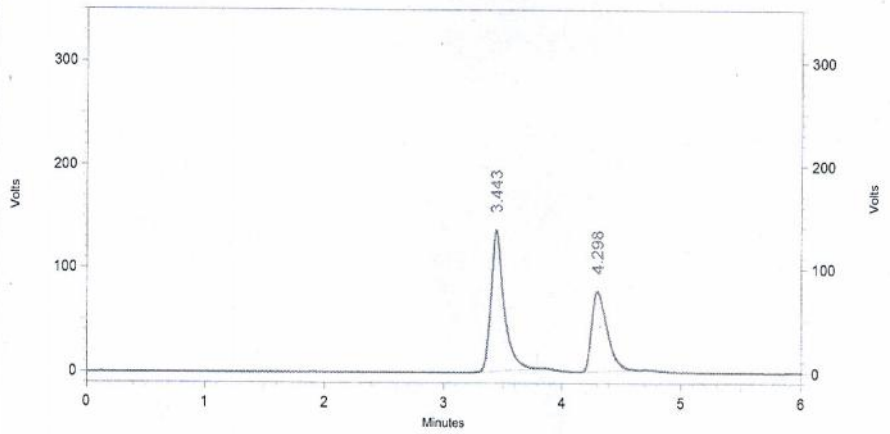
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
CEFPODOXIME	3.443	1028274	59.80	135149	63.96	1.52	5223	0.00
AMBROXAL	4.298	691211	40.20	76145	36.04	1.67	4982	3.94
Totals		1719485	100.00	211294	100.00			

Figure-37

CHROMATOGRAM FOR ANALYSIS OF FOMULATION FINECEF-AM TAB FOR LOW LEVEL DILUTONS REPEATABILITY - 2

Area % Report

Description SPL- 02_FORMULATION - LOW LEVEL CONCENTRATION - 80%
Data File: D:\ChromQuest\PROJECTS\DATA\CEFPODOXIME + AMBROXOL\18.01.2012\015_PA_L-02.dat
Method: D:\ChromQuest\Method\CEFPODOXIME + AMBROXOL.met
Acquired: 1/18/2012 6:46:15 PM
Wave Length 247 nm



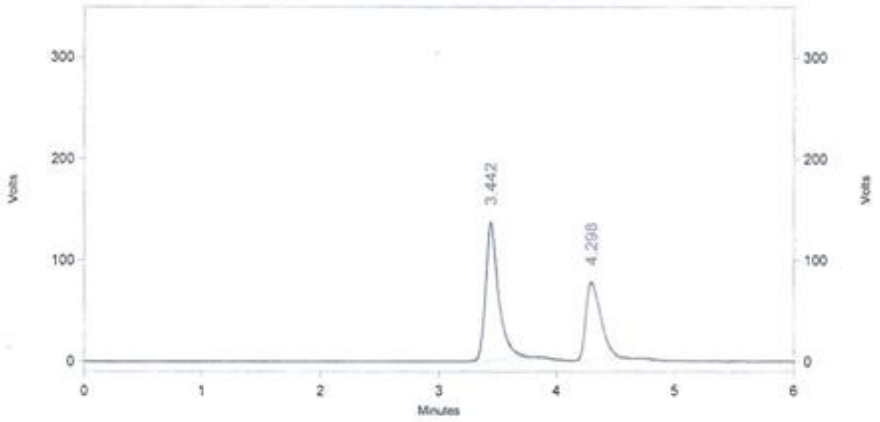
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
CEFPODOXIME	3.443	1027615	59.81	135490	63.83	1.50	5224	0.00
AMBROXOL	4.298	690576	40.19	76793	36.17	1.66	5060	3.96
Totals		1718191	100.00	212283	100.00			

Figure-38

CHROMATOGRAM FOR ANALYSIS OF FOMULATION FINECEF-AM TAB FOR LOW LEVEL DILUTONS REPEATABILITY - 3

Area % Report

Description SPL- 03_ -FORMULATION - LOW LEVEL CONCENTRATION - 80%
Data File: D:\ChromQuest\PROJECTS\DATA\CEFPODOXIME + AMBROXOL\18.01.2012\016_PA_L-03.dat
Method: D:\ChromQuest\Method\CEFPODOXIME + AMBROXOL.met
Acquired: 1/18/2012 6:55:24 PM
Wave Length 247 nm



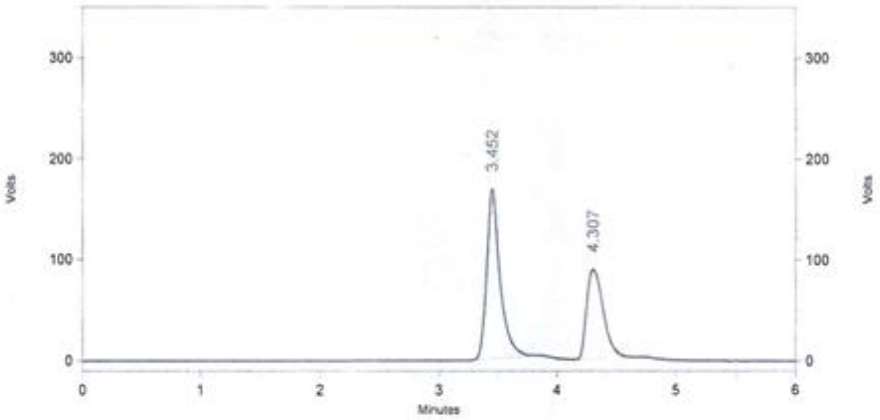
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
CEFPODOXIME	3.442	1032013	59.90	135655	63.89	1.52	5214	0.00
AMBROXOL	4.298	690886	40.10	76674	36.11	1.65	5030	3.96
Totals		1722899	100.00	212329	100.00			

Figure-39

CHROMATOGRAM FOR ANALYSIS OF FOMULATION FINECEF-AM TAB FOR MID LEVEL DILUTONS REPEATABILITY – 1

Area % Report

Description SPL- 01- FORMULATION - MIDDLE LEVEL CONCENTRATION - 100%
Data File: D:\ChromQuest\PROJECTS\DATA\CEFPODOXIME + AMBROXOL\18.01.2012\017_PA_M-01.dat
Method: D:\ChromQuest\Method\CEFPODOXIME + AMBROXOL.met
Acquired: 1/18/2012 7:04:31 PM
Wave Length 247 nm



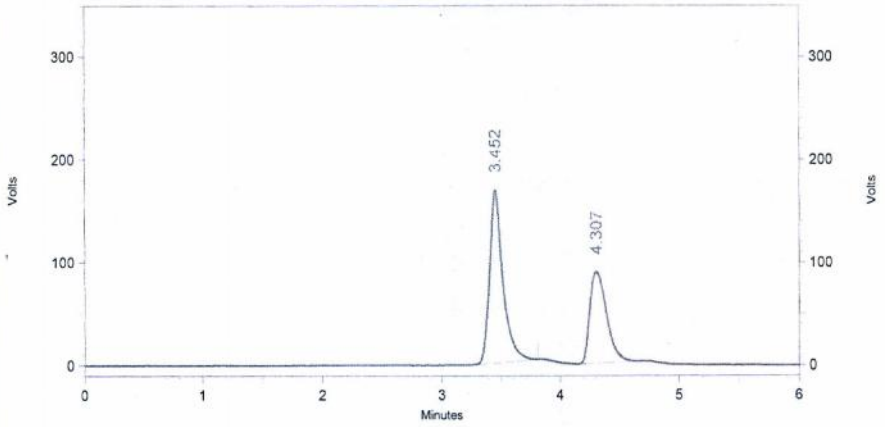
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
CEFPODOXIME	3.452	1311080	59.86	168143	65.49	1.44	4905	0.00
AMBROXOL	4.307	879163	40.14	88596	34.51	1.53	4610	3.79
Totals		2190243	100.00	256739	100.00			

Figure-40

CHROMATOGRAM FOR ANALYSIS OF FOMULATION FINECEF-AM TAB FOR MID LEVEL DILUTONS REPEATABILITY - 2

Area % Report

Description SPL- 02_FORMULATION - MIDDLE LEVEL CONCENTRATION - 100%
Data File: D:\ChromQuest\PROJECTS\DATA\CEFPODOXIME + AMBROXOL\18.01.2012\018_PA_M-02.dat
Method: D:\ChromQuest\Method\CEFPODOXIME + AMBROXOL.met
Acquired: 1/18/2012 7:13:39 PM
Wave Length 247 nm



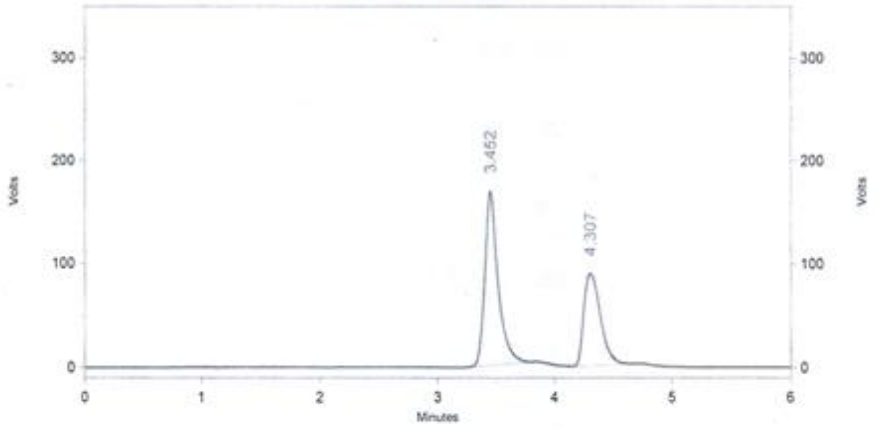
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
CEFPODOXIME	3.452	1309291	59.84	167923	65.46	1.44	4905	0.00
AMBROXOL	4.307	878809	40.16	88618	34.54	1.53	4612	3.79
Totals		2188100	100.00	256541	100.00			

Figure-41

CHROMATOGRAM FOR ANALYSIS OF FOMULATION FINECEF-AM TAB FOR MID LEVEL DILUTONS REPEATABILITY – 3

Area % Report

Description SPL- 03_FORMULATION - MIDDLE LEVEL CONCENTRATION - 100%
Data File: D:\ChromQuest\PROJECTS\DATA\CEFPODOXIME + AMBROXOL\18.01.2012\019_PA_M-03.dat
Method: D:\ChromQuest\Method\CEFPODOXIME + AMBROXOL.met
Acquired: 1/18/2012 7:22:47 PM
Wave Length 247 nm



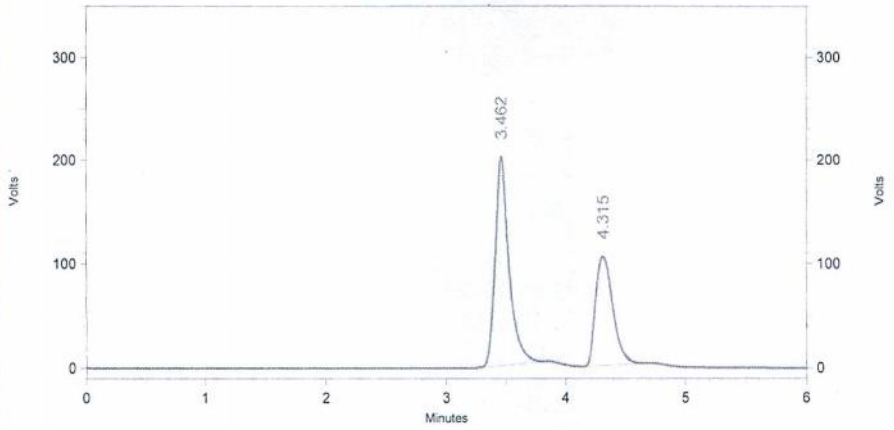
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
CEFPODOXIME	3.452	1312563	59.85	168101	65.52	1.44	4880	0.00
AMBROXOL	4.307	880609	40.15	88454	34.48	1.52	4595	3.79
Totals		2193172	100.00	256555	100.00			

Figure-42

CHROMATOGRAM FOR ANALYSIS OF FOMULATION FINECEF-AM TAB FOR HIGH LEVEL DILUTONS REPEATABILITY – 1

Area % Report

Description SPL- 01_FORMULATION - HIGH LEVEL CONCENTRATION - 120%
Data File: D:\ChromQuest\PROJECTS\DATA\CEFPODOXIME + AMBROXOL\18.01.2012\020_PA_H-01.dat
Method: D:\ChromQuest\Method\CEFPODOXIME + AMBROXOL.met
Acquired: 1/18/2012 7:31:55 PM
Wave Length 247 nm



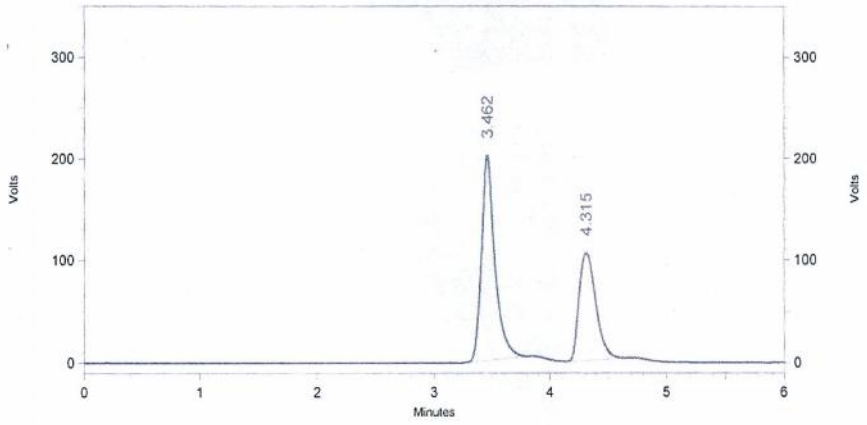
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
CEFPODOXIME	3.462	1600372	59.90	200532	65.77	1.36	4647	0.00
AMBROXOL	4.315	1071428	40.10	104388	34.23	1.38	4345	3.67
Totals		2671800	100.00	304920	100.00			

Figure-43

CHROMATOGRAM FOR ANALYSIS OF FOMULATION FINECEF-AM TAB FOR HIGH LEVEL DILUTONS REPEATABILITY - 2

Area % Report

Description SPL- 02_FORMULATION - HIGH LEVEL CONCENTRATION - 120%
Data File: D:\ChromQuest\PROJECTS\DATA\CEFPODOXIME + AMBROXOL\18.01.2012\021_PA_H-02.dat
Method: D:\ChromQuest\Method\CEFPODOXIME + AMBROXOL.met
Acquired: 1/18/2012 7:41:02 PM
Wave Length 247 nm



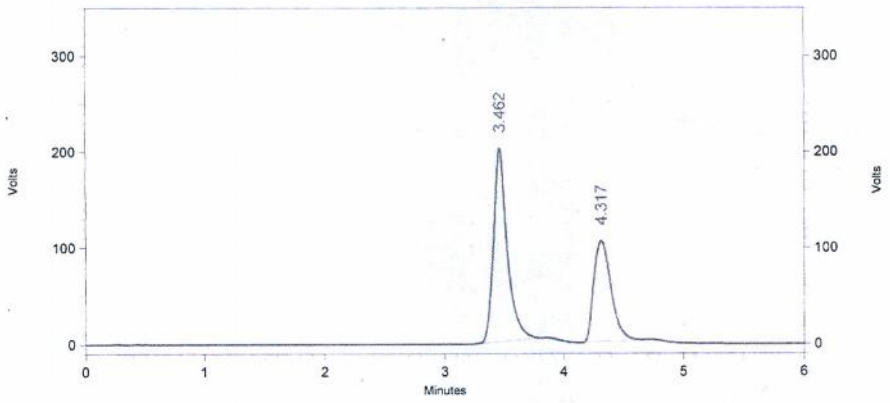
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
CEFPODOXIME	3.462	1596522	59.92	200591	65.77	1.36	4666	0.00
AMBROXOL	4.315	1068021	40.08	104408	34.23	1.39	4378	3.68
Totals		2664543	100.00	304999	100.00			

Figure-44

CHROMATOGRAM FOR ANALYSIS OF FOMULATION FINECEF-AM TAB FOR HIGH LEVEL DILUTONS REPEATABILITY - 3

Area % Report

Description SPL- 03_FORMULATION - HIGH LEVEL CONCENTRATION - 120%
Data File: D:\ChromQuest\PROJECTS\DATA\CEFPODOXIME + AMBROXOL\18.01.2012\022_PA_H-03.dat
Method: D:\ChromQuest\Method\CEFPODOXIME + AMBROXOL.met
Acquired: 1/18/2012 7:50:10 PM
Wave Length 247 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
CEFPODOXIME	3.462	1597931	59.91	200564	65.81	1.36	4660	0.00
AMBROXOL	4.317	1069080	40.09	104201	34.19	1.37	4354	3.68
Totals		2667011	100.00	304765	100.00			

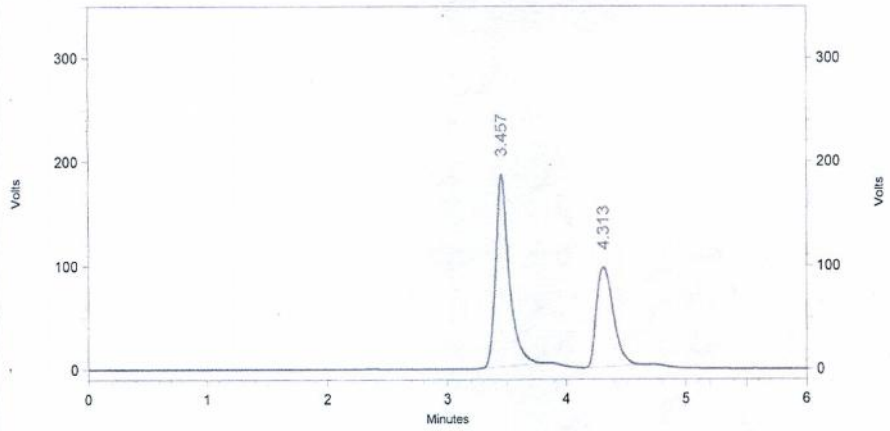
Figure-45

CHROMATOGRAM FOR 110% RECOVERY OF FORMULATION

FINECEF-AM TAB

Area % Report

Description SPL-RECOVERY- 01_FORMULATION - STANDARD 10%
Data File: D:\ChromQuest\PROJECTS\DATA\CEFPODOXIME + AMBROXOL\18.01.2012\023_RECOVERY-110%.dat
Method: D:\ChromQuest\Method\CEFPODOXIME + AMBROXOL.met
Acquired: 1/18/2012 7:59:18 PM
Wave Length 247 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
CEFPODOXIME	3.457	1451500	59.87	184883	65.37	1.39	4804	0.00
AMBROXOL	4.313	973091	40.13	97932	34.63	1.43	4540	3.76
Totals		2424591	100.00	282815	100.00			

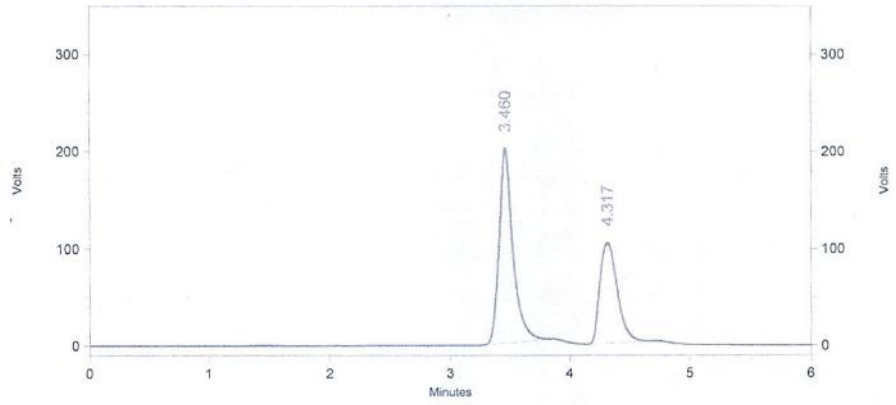
Figure-46

CHROMATOGRAM FOR 120% RECOVERY OF FORMULATION

FINECEF-AM TAB

Area % Report

Description SPL-RECOVERY- 02_FORMULATION - STANDARD 20%
Data File: D:\ChromQuest\PROJECTS\DATA\CEFPODOXIME + AMBROXOL\18.01.2012\024_RECOVERY-120%.dat
Method: D:\ChromQuest\Method\CEFPODOXIME + AMBROXOL.met
Acquired: 1/18/2012 8:08:25 PM
Wave Length 247 nm



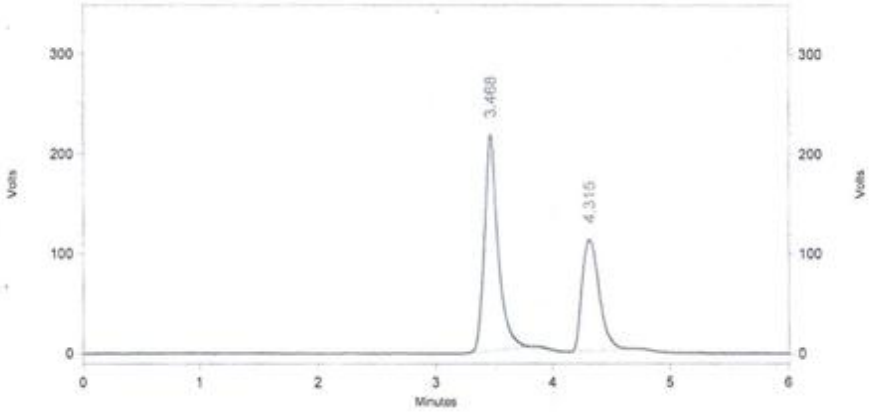
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
CEFPODOXIME	3.460	1598559	59.95	200371	65.41	1.37	4664	0.00
AMBROXOL	4.317	1067752	40.05	105957	34.59	1.37	4357	3.69
Totals		2666311	100.00	306328	100.00			

Figure-47

CHROMATOGRAM FOR 130% RECOVERY OF FOMULATION FINECEF-AM TAB

Area % Report

Description SPL-RECOVERY- 03_FORMULATION- STANDARD 30%
Data File: D:\ChromQuest\PROJECTS\DATA\CEFPODOXIME + AMBROXOL\18.01.2012\025_RECOVERY-130%.dat
Method: D:\ChromQuest\Method\CEFPODOXIME + AMBROXOL.met
Acquired: 1/18/2012 8:17:34 PM
Wave Length 247 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
CEFPODOXIME	3.460	1753341	59.86	220148	65.34	1.36	4664	0.00
AMBROXOL	4.317	1175584	40.14	116796	34.66	1.37	4375	3.69
Totals		2928925	100.00	336944	100.00			

TABLES

TABLE-1

SOLUBILITY PROFILE OF CEFPODOXIME PROXETIL AND AMBROXOL HCl IN POLAR AND NON POLAR SOLVENTS

S.NO	SOLVENTS	CEFPODOXIME PROXETIL		AMBROXOL HYDROCHLORIDE	
		SOLUBILITY	CATEGORY	SOLUBILTY	CATEGORY
1	Distilled water	10mg in 100ml	Very slightly soluble	10mg in 0.6ml	Sparingly soluble
2	0.1 M Hydrochloric acid	More than 100ml	Practically insoluble	10mg in 1ml	Sparingly soluble
3	0.1 M Sodium Hydroxide	More than 100ml	Practically insoluble	10mg in 100ml	Very slightly soluble
4	Methanol	10mg in 0.1ml	Freely soluble	10mg in 0.08ml	Freely soluble
5	Ethanol	10mg in 0.1ml	Freely soluble	10mg in 0.5ml	Sparingly soluble
6	Chloroform	10mg in 30ml	Very slightly soluble	More than 100ml	Practically insoluble
7	Di methyl formamide	10mg in 0.05ml	Freely soluble	10mg in 0.06ml	Freely soluble
8	Acetone	10mg in 0.05ml	Freely soluble	10mg in 100ml	Very slightly soluble
9	Toluene	10mg in 50ml	Very slightly soluble	More than 100ml	Practically insoluble
10	Benzene	10mg in 50ml	Very slightly soluble	More than 100ml	Practically insoluble
11	n –Butanol	10mg in 0.3ml	soluble	10mg in 8ml	Slightly soluble
12	Acetonitrile	10mg in 0.05ml	Freely soluble	10mg in 100ml	Very slightly soluble
13	Hexane	10mg in 100ml	Very slightly soluble	More than 100ml	Practically insoluble
14	Di ethyl ether	10mg in 15ml	Very slightly soluble	More than 100ml	Practically insoluble
15	Isopropyl alcohol	10mg in 0.25ml	Soluble	10mg in 5ml	Slightly soluble
16	Di chloro methane	10mg in 2ml	Slightly soluble	More than 100ml	Practically insoluble
17	Acetic acid	10mg in 0.1ml	Freely soluble	10mg in 1ml	Sparingly soluble
18	Ethyl acetate	10mg in 0.15ml	Soluble	10mg in 50ml	Very slightly soluble
19	Phosphate buffer pH 3	10mg in 100ml	Very slightly soluble	10mg in 1ml	Sparingly soluble
20	Phosphate buffer pH 5	More than 100ml	Practically insoluble	10mg in 5ml	Slightly soluble
21	Phosphate buffer pH 7	More than 100ml	Practically insoluble	10mg in 7ml	Slightly soluble
22	Phosphate buffer pH 9	More than 100ml	Practically insoluble	10mg in 15ml	Very slightly soluble

TABLE-2

OPTICAL CHARACTERSTICS OF CEFPODOXIME PROXETIL BY SIMULTANEOUS EQUATION METHOD (235, 248, 308 nm)

PARAMETERS	AT 235 nm*	AT 248 nm*	AT 308 nm*
Beers law limit ($\mu g\ mL^{-1}$)	5-30 ($\mu g\ mL^{-1}$)	5-30 ($\mu g\ mL^{-1}$)	5-30 ($\mu g\ mL^{-1}$)
Molar absorptivity ($Lmol^{-1}cm^{-1}$)	18470.06786	16474.45469	51116.30408
Sandells sensitivity ($\mu g/cm^2$/0.001 A.U)	0.029948177	0.033581809	0.108832656
Correlation coefficient (r)	0.99988	0.99989	0.99980
Regression equation (y= mx + c)	Y= (0.03342)x + (-0.00297)	Y= (0.02980)x + (-0.00259)	Y= (0.00922)x + (-0.000447)
Slope (m)	0.0334208	0.0298042	0.009220119
Intercept (c)	-0.0029720	-0.0025952	-0.000447024
LOD ($\mu g\ mL^{-1}$)	0.3230845	0.2335186	0.47452324
LOQ ($\mu g\ mL^{-1}$)	0.979043	0.707632	1.437949215
Standard error	0.0056158	0.0047849	0.00212772
Absorptivity $A^{1\%}_{1cm}$	331.833	295.767	91.78546

*Mean of six observations

TABLE-3

OPTICAL CHARACTERSTICS OF AMBROXOL HYDROCHLORIDE BY SIMULTANEOUS EQUATION METHOD (235, 248, 308 nm)

PARAMETERS	AT 235 nm*	AT 248 nm*	AT 308 nm*
Beers law limit (µg mL-1)	3-18 (µg mL-1)	3-18 (µg mL-1)	3-18 (µg mL-1)
Molar absorptivity (Lmol-1cm-1)	7499.1844	12068.0928	3693.7898
Sandells sensitivity (µg/cm2/0.001 A.U)	0.056537089	0.034806494	0.115827419
Correlation coefficient (r)	0.99958	0.99973	0.99976
Regression equation (y= mx + c)	Y= (0.01797)x + (0.001149)	Y= (0.029111)x + (0.000035119)	Y= (0.087642)x + (0.00145)
Slope (m)	0.017972817	0.02911131	0.00876428
Intercept (c)	0.001149405	0.000035119	0.00145
LOD (µg mL-1)	0.537561213	0.45856963	0.354468818
LOQ (µg mL-1)	1.628973373	1.389604939	1.0741479
Standard error	0.003563439	0.004304659	0.00129398
Absorptivity ($A^{1\%}_{1cm}$)	182.2937	290.7699	90.24922

*Mean of six observations

TABLE-4

QUANTIFICATION OF TABLET FORMULATION (FINECEF-AM TAB)

BY SIMULTANEOUS EQUATION METHOD (235, 248 nm)

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained* (%)	Average (%)	S.D	% R.S.D.	S.E.
CEF	1	100	99.9368	99.94	99.8733	0.08891	0.08903	0.0247
	2	100	99.7853	99.79				
	3	100	99.8000	99.80				
	4	100	99.7853	99.79				
	5	100	99.9368	99.94				
	6	100	99.9850	99.98				
AMB	1	60	60.5909	100.98	101.0917	0.16179	0.16004	0.0044
	2	60	60.8900	101.30				
	3	60	60.6109	101.01				
	4	60	60.8900	101.30				
	5	60	60.5909	100.98				
	6	60	60.5909	100.98				

***Mean of six observations

TABLE - 5

QUANTIFICATION OF TABLET FORMULATION (FINECEF-AM TAB)

BY SIMULTANEOUS EQUATION METHOD (235, 308 nm)

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained* (%)	Average (%)	S.D	% R.S.D.	S.E.
CEF	1	100	101.6847	101.68	101.6467	0.0816	0.08032	0.0226
	2	100	101.6847	101.68				
	3	100	101.4847	101.48				
	4	100	101.6847	101.68				
	5	100	101.6847	101.68				
	6	100	101.6847	101.68				
AMB	1	60	59.8140	99.69	99.5883	0.3009	0.30220	0.0083
	2	60	59.6110	99.35				
	3	60	60.0609	100.10				
	4	60	59.6110	99.35				
	5	60	59.8140	99.69				
	6	60	59.6110	99.35				

***Mean of six observations

TABLE 6

INTRA DAY AND INTER DAY ANALYSIS OF FORMULATION BY SIMULTANEOUS EQUATION METHOD (235,248 nm)

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained* (%)		S.D		% R.S.D.	
			Intra day	Inter day	Intra day	Inter Day	Intra day	Inter day
CEF	1	100	99.87	99.87	0.02081	0.0583	0.0208	0.5828
	2	100	99.84	100.86				
	3	100	99.88	99.81				
Mean			99.86	99.87				
AMB	1	60	101.09	101.09	0.07630	0.4020	0.0755	0.3985
	2	60	100.19	100.42				
	3	60	101.04	101.14				
MEAN			101.10	100.69				

*Mean of Three observations

TABLE 7

INTRA DAY AND INTER DAY ANALYSIS OF FORMULATION BY SIMULTANEOUS EQUATION METHOD (235,308 nm)

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained* (%)		S.D		% R.S.D.	
			Intra day	Inter day	Intra day	Inter day	Intra day	Inter day
CEF	1	100	101.64	101.64	0.01732	0.9588	0.0170	0.9488
	2	100	101.61	99.95				
	3	100	101.64	101.58				
Mean			101.63	101.05				
AMB	1	60	99.65	99.58	0.0916	0.1677	0.0919	0.1682
	2	60	99.71	99.86				
	3	60	99.53	99.56				
MEAN			99.63	99.66				

*Mean of Three observations

TABLE-8

RUGGEDNESS STUDY BY SIMULTANEOUS EQUATION METHOD

(235, 248 nm)

Drug	Condition	Average* % Obtained	S.D	% R.S.D	S.E.
CEF	Analyst 1	99.8733	0.088919	0.089032	0.0247
	Analyst 2	99.8550	0.070356	0.070458	0.001954
	Instrument 1	99.8133	0.088694	0.088862	0.002464
	Instrument 2	100.9233	0.12242	0.1213	0.003401
AMB	Analyst 1	101.0917	0.161792	0.160045	0.004494
	Analyst 2	101.0533	0.193149	0.191136	0.005365
	Instrument 1	101.2233	0.405446	0.400546	0.011262
	Instrument 2	100.2833	0.268527	0.267768	0.007459

TABLE-9

RUGGEDNESS STUDY BY SIMULTANEOUS EQUATION METHOD

(235, 308 nm)

Drug	Condition	Average* % Obtained	S.D	% R.S.D	S.E.
CEF	Analyst 1	101.6467	0.08165	0.080327	0.002268
	Analyst 2	101.4833	0.08165	0.080456	0.002268
	Instrument 1	101.5833	0.10328	0.10167	0.002869
	Instrument 2	100.1700	0.972091	0.970441	0.027003
AMB	Analyst 1	99.5883	0.30096	0.302204	0.00836
	Analyst 2	99.9366	0.302104	0.302295	0.008392
	Instrument 1	99.5666	0.382134	0.383797	0.010615
	Instrument 2	99.8933	0.686809	0.687542	0.019078

TABLE 10

RECOVERY STUDY DATA OF 50% PRE ANALYSED FORMULATION

BY SIMULTANEOUS EQUATION METHOD (235, 248 nm)

Drug	%	Amount present* ($\mu g\ mL^{-1}$)	Amount added* ($\mu g\ mL^{-1}$)	Amount estimated* ($\mu g\ mL^{-1}$)	Amount recovered* ($\mu g\ mL^{-1}$)	% Recovery*	S.D.	% RSD	S.E.
CEF	80	10	8	17.9862	7.9862	100.1721	0.1506	0.1502	0.0167
	100	10	10	19.9557	9.9557	100.4400			
	120	10	12	21.9775	11.9775	100.1866			
					Mean	100.2662			
AMB	80	6	4.8	10.8500	4.8500	98.9700	0.3807	0.3830	0.0423
	100	6	6	12.0313	6.0313	99.5023			
	120	6	7.2	13.2212	7.2212	99.7077			
					Mean	99.3933			

*Mean of Three observations

TABLE 11

RECOVERY STUDY DATA OF 50% PRE ANALYSED FORMULATION

BY SIMULTANEOUS EQUATION METHOD (235, 308 nm)

Drug	%	Amount present* ($\mu g\ mL^{-1}$)	Amount added* ($\mu g\ mL^{-1}$)	Amount estimated* ($\mu g\ mL^{-1}$)	Amount recovered* ($\mu g\ mL^{-1}$)	% Recovery*	S.D.	% RSD	S.E.
CEF	80	10	8	18.0300	8.0300	99.6217	0.3575	0.3601	0.0397
	100	10	10	20.1100	10.1100	98.9120			
	120	10	12	22.0816	12.0816	99.3430			
					Mean	99.2922			
AMB	80	6	4.8	10.7710	4.7715	100.2610	0.1180	0.1175	0.0131
	100	6	6	11.9820	5.9823	100.4970			
	120	6	7.2	13.1633	7.1633	100.3773			
					Mean	100.3784			

*Mean of Three observations

TABLE-12

OPTICAL CHARACTERSTICS OF CEFPODOXIME PROXETIL BY AREA UNDER THE CURVE METHOD

PARAMETERS	AT 229-238 nm*	AT 291-316 nm*
Beers law limit (μg mL^{-1})	5-30 (μg mL^{-1})	5-30 (μg mL^{-1})
Molar absorptivity ($Lmol^{-1}cm^{-1}$)	164681.6166	156282.1181
Sandells sensitivity (μg/cm^2/0.001 A.U)	0.00339971	0.00356851
Correlation coefficient (r)	0.99994	0.99993
Regression equation (y= mx + c)	Y= (0.2941)x +(0.011838)	Y=(0.28034)x+(-0.000744)
Slope (m)	0.294150952	0.280345833
Intercept (c)	0.011838095	-0.000744643
LOD (μg mL^{-1})	0.184128613	0.347769322
LOQ (μg mL^{-1})	0.557965495	1.053846432
Standard error	0.032160772	0.037751137
Absorptivity ($A^{1\%}_{1cm}$)	2955.6756	2802.0336

*Mean of six observations

TABLE-13

OPTICAL CHARACTERSTICS OF AMBROXOL HYDROCHLORIDE BY AREA UNDER THE CURVE METHOD

PARAMETERS	AT 229-238 nm*	AT 291-316 nm*
Beers law limit (µg mL^{-1})	3-18 (µg mL^{-1})	3-18 (µg mL^{-1})
Molar absorptivity ($Lmol^{-1}cm^{-1}$)	85223.05364	86867.92156
Sandells sensitivity (µg/cm^2/0.001 A.U)	0.004854153	0.004773401
Correlation coefficient (r)	0.99993	0.99989
Regression equation (y= mx + c)	Y= (0.20602)x +(0.000063)	Y=(0.20957)x+(-0.000573)
Slope (m)	0.206027976	0.209579563
Intercept (c)	0.0000636905	-0.000573214
LOD (µg mL^{-1})	0.162339083	0.198614195
LOQ (µg mL^{-1})	0.491936622	0.6018611960
Standard error	0.015121428	0.019799428
Absorptivity ($A_{1cm}^{1\%}$)	2065.7850	2095.0691

*Mean of six observations

TABLE 14

QUANTIFICATION OF TABLET FORMULATION (FINECEF-AM TAB)

BY AREA UNDER THE CURVE METHOD

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained* (%)	Average (%)	S.D	% R.S.D.	S.E.
CEF	1	100	99.7050	99.70	99.73	0.07099	0.07118	0.00197
	2	100	99.6758	99.67				
	3	100	99.6758	99.67				
	4	100	99.8220	99.82				
	5	100	99.7050	99.70				
	6	100	99.8220	99.82				
AMB	1	60	60.3900	100.65	100.48	0.15205	0.15132	0.00422
	2	60	60.2900	100.48				
	3	60	60.2900	100.48				
	4	60	60.1900	100.31				
	5	60	60.3900	100.65				
	6	60	60.1900	100.31				

*Mean of six observations

TABLE 15

INTRA DAY AND INTER DAY ANALYSIS OF FORMULATION BY AREA UNDER THE CURVE METHOD

Drug	Sample No.	Labeled amount (mg/tab)	Percentage Obtained* (%)		S.D		% R.S.D.	
			Intra day	Inter day	Intra day	Inter day	Intra day	Inter day
CEF	1	100	99.73	99.73	0.0173	0.0305	0.0173	0.0306
	2	100	99.73	99.69				
	3	100	99.70	99.67				
MEAN			99.7200	99.6766				
AMB	1	60	100.42	100.48	0.0346	0.0665	0.0344	0.0662
	2	60	100.48	100.59				
	3	60	100.48	100.47				
MEAN			100.460	100.513				

*Mean of Three observations

TABLE 16

RUGGEDNESS STUDY BY AREA UNDER THE CURVE METHOD

Drug	Condition	Average* % Obtained	S.D	% R.S.D	S.E.
CEF	Analyst 1	99.7300	0.070993	0.071185	0.001972
	Analyst 2	99.6700	0.06000	0.060199	0.001667
AMB	Analyst 1	100.4800	0.152053	0.151326	0.004224
	Analyst 2	100.4483	0.104387	0.103921	0.002900

TABLE 17

RECOVERY STUDY DATA OF 50% PRE ANALYSED FORMULATION

BY AREA UNDER THE CURVE METHOD

Drug	%	Amount present* ($\mu g\ mL^{-1}$)	Amount added* ($\mu g\ mL^{-1}$)	Amount estimated* ($\mu g\ mL^{-1}$)	Amount recovered* ($\mu g\ mL^{-1}$)	% Recovery*	S.D.	% R.S.D.	S.E.
CEF	80	10	8	17.9600	7.9600	100.5008	0.0298	0.0297	0.0033
	100	10	10	19.9534	9.9534	100.4580			
	120	10	12	21.9462	11.9462	100.4433			
					Mean	100.4674			
AMB	80	6	4.8	10.8300	4.8300	99.3700	0.2673	0.2691	0.0297
	100	6	6	12.0266	6.0266	99.5574			
	120	6	7.2	13.2700	7.2700	99.0300			
					Mean	99.3191			

*Mean of Three observations

TABLE-18

OPTICAL CHARACTERSTICS OF CEFPODOXIME PROXETIL AND AMBROXOL HYDROCHLORIDE BY DERIVATIVE SPECTROSCOPIC METHOD

PARAMETERS	CEFPODOXIME AT 279 nm*	AMBROXOL AT 235 nm*
Beers law limit (µg mL^{-1})	10-60 (µg mL^{-1})	6-36 (µg mL^{-1})
Molar absorptivity ($Lmol^{-1}cm^{-1}$)	194.56606	242.87007861
Sandells sensitivity (µg/cm^2/0.001 A.U)	2.799078	1.788869362
Correlation coefficient (r)	0.99964	0.99949
Regression equation (y= mx + c)	Y = (0.0003573)x + (-0.0000839)	Y = (0.0005903)x + (-0.0000458333)
Slope (m)	0.000357321	0.000590377
Intercept (c)	-0.0000839286	-0.0000458333
LOD (µg mL^{-1})	0.046913171	1.357334448
LOQ (µg mL^{-1})	0.142161124	4.113134692
Standard error	0.00020266	0.000252753
Absorptivity ($A_1^{1\%}{}_{1cm}$)	3.534583292	5.865740833

*Mean of six observations

TABLE-19

QUANTIFICATION OF TABLET FORMULATION (FINEEF-AM TAB)

BY DERIVATIVE SPECTROSCOPIC METHOD

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D.	S.E.
CEF	1	100	100.1849	100.18	99.9423	0.40625	0.40648	0.011285
	2	100	100.1849	100.18				
	3	100	99.4050	99.40				
	4	100	100.1849	100.18				
	5	100	99.4400	99.44				
	6	100	100.2740	100.27				
AMB	1	60	60.7408	101.16	101.77	0.60643	0.59588	0.01684
	2	60	60.5900	102.50				
	3	60	60.9900	101.65				
	4	60	60.7408	101.16				
	5	60	60.9900	101.65				
	6	60	61.5900	102.5				

*Mean of six observations

TABLE 20

INTRA DAY AND INTER DAY ANALYSIS OF FORMULATION BY DERIVATIVE SPECTROSCOPIC METHOD

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.	
			Intra day	Inter day	Intra day	Inter day	Intra day	Inter day
CEF	1	100	99.89	99.81	0.3666	0.1973	0.3685	0.1981
	2	100	99.20	99.49				
	3	100	99.33	99.45				
MEAN			99.72	99.67				
AMB	1	60	100.42	100.48	0.6365	0.6702	0.6350	0.6642
	2	60	100.48	100.59				
	3	60	100.48	100.47				
MEAN			100.46	100.51				

*Mean of Three observations

TABLE 21

RUGGEDNESS STUDY BY DERIVATIVE SPECTROSCOPIC METHOD

Drug	Condition	Average* % Obtained	S.D	% R.S.D	S.E.
CEF	Analyst 1	99.9423	0.406252	0.406486	0.011285
	Analyst 2	99.2966	0.228619	0.230238	0.006351
AMB	Analyst 1	101.7700	0.606432	0.595885	0.016845
	Analyst 2	100.5800	0.898532	0.893351	0.024959

TABLE-22

RECOVERY STUDY DATA OF 50% PRE ANALYSED FORMULATION

BY DERIVATIVE SPECTROSCOPIC METHOD

Drug	%	Amount present* ($\mu g\ mL^{-1}$)	Amount added* ($\mu g\ mL^{-1}$)	Amount estimated* ($\mu g\ mL^{-1}$)	Amount recovered* ($\mu g\ mL^{-1}$)	% Recovery*	S.D.	% R.S.D.	S.E.
CEF	80	10	8	17.9060	7.9060	101.1861	0.2028	0.2006	0.0225
	100	10	10	19.9153	9.9153	100.8605			
	120	10	12	21.8539	11.8539	100.2330			
					Mean	101.0932			
AMB	80	6	4.8	10.8050	4.8050	99.9359	0.2379	0.2378	0.0264
	100	6	6	12.0099	6.0099	99.8400			
	120	6	7.2	13.1800	7.1800	100.2916			
					Mean	100.0225			

*Mean of Three observations

TABLE - 23

SYSTEM SUITABILITY PARAMETERS FOR THE OPTIMIZED CHROMATOGRAM BY RP - HPLC

PARAMETERS	CEFPODOXIME PROXETIL	AMBROXOL HYDROCHLORIDE
Tailing factor	1.14	1.16
Asymmetrical factor	1.44	1.49
Theoretical plates	4962	4637
Capacity factor	3.32125	4.385
Theoretical plate per unit Length	330.8	309.133
Resolution	Between CEF and AMB 3.79	

TABLE-24

OPTICAL CHARACTERISTICS OF CEFPODOXIME PROXETIL AND AMBROXOL HYDROCHLORIDE BY RP - HPLC

PARAMETERS	CEFPODOXIME PROXETIL*	AMBROXOL HCl*
Detection wavelength (nm)	247	247
Beers law limit (μg mL^{-1})	70-130 (μg mL^{-1})	42-78 (μg mL^{-1})
Correlation coefficient (r)	0.9997	0.9999
Régression équation (y=mx+c)	Y= (14303.1107)x+ (- 121339.6429)	Y=(14879.4881)x+ (-24882.5714)
Slope (m)	14303.1107	14879.4881
Intercept (c)	-121339.6429	-24882.5714
LOD (μg mL^{-1})	0.000.37747	0.00054925
LOQ (μg mL^{-1})	0.001326507	0.001664394
Standard Error	0.60014693	1.093552806

*Mean of Three observations

TABLE -25

QUANTIFICATION OF TABLET FORMULATION (FINECEF-AM TAB)

BY RP-HPLC

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)	Percentage Obtained* (%)	Average (%)	S.D.	% R.S.D.	S.E.
CEF	L 1	100	99.04	99.04	99.18	0.2100	0.2100	0.0225
	O 2	100	99.08	99.08				
	W 3	100	99.41	99.41				
	M 1	100	99.22	99.22	99.27	0.1400	0.1400	0.0153
	I 2	100	99.17	99.17				
	D 3	100	99.43	99.43				
	H 1	100	99.14	99.14	99.15	0.0903	0.0903	0.01800
	I 2	100	99.07	99.07				
	G 3	100	99.25	99.25				
	H							
AMB	L 1	60	59.91	99.85	99.85	0.0251	0.0252	0.0279
	O 2	60	59.92	99.87				
	W 3	60	59.89	99.82				
	M 1	60	59.88	99.80	99.90	0.1422	0.1423	0.0158
	I 2	60	59.90	99.83				
	D 3	60	60.03	100.06				
	H 1	60	59.73	99.55	99.52	0.1041	0.1045	0.0115
	I 2	60	59.64	99.40				
	G 3	60	59.76	99.60				
	H							

* Mean of six observations

TABLE-26

RECOVERY STUDIES OF 50% PREANALYZED FORMULATION

(FINECEF-AM TAB) BY RP-HPLC

Drug	%	Amount present* (µg mL^{-1})	Amount added* (µg mL^{-1})	Amount estimated* (µg mL^{-1})	Amount recovered* (µg mL^{-1})	% Recovery*	S.D.	% R.S.D.	S.E.
CEF	80	100	10	109.13	9.9290	99.29	0.6878	0.6885	0.0764
	100	100	20	119.33	20.1310	100.65			
	120	100	30	129.14	29.9360	99.79			
					Mean	99.91			
AMB	80	60	6	65.84	5.9880	99.81	0.6987	0.7006	0.0776
	100	60	12	71.73	11.8790	98.99			
	120	60	18	77.92	18.0680	100.38			
					Mean	99.72			

*Mean of Three observations

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