METHOD DEVELOPMENT AND VALIDATION OF NEWER ANALYTICAL TECHNIQUES

Ph.D. thesis Submitted to

The Tamil Nadu Dr. M.G.R. Medical University,
Chennai - 600 032, Tamilnadu, India.

In partial fulfilment for the award of Degree of
DOCTOR OF PHILOSOPHY
(Faculty of Pharmacy)

Submitted by
G. ABIRAMI, M.Pharm.

Under the Guidance of
Dr. T. VETRICHELVAN, M.Pharm., Ph.D.
Principal and Head
(Department of Pharmaceutical Analysis)

ADHIPARASAKTHI COLLEGE OF PHARMACY
(Accredited by "NAAC" with CGPA of 2.74 on a four point scale at "B" Grade)
MELMARUVATHUR – 603 319

SEPTEMBER - 2013

DECLARATION

I hereby declare that the thesis entitled **"METHOD DEVELOPMENT AND VALIDATION OF NEWER ANALYTICAL TECHNIQUES"** submitted by me, as a partial fulfilment for the award of Degree of Doctor of Philosophy (Faculty of Pharmacy) is a record of research work done by me during 2008 to 2013, under the guidance and supervision of **Dr. T. VETRICHELVAN, M. Pharm, Ph.D.,** Head and Principal of Adhiparasakthi College of Pharmacy, Melmaruvathur, TamilNadu, India, and has not formed the basis for the award of any other degree, diploma, associateship, fellowship or any other similar title to any other university or similar institute of higher learning.

Place: Melmaruvathur
Date:

Mrs. G.ABIRAMI, M. Pharm.,
Assistant Professor,
Adhiparasakthi College of Pharmacy,
Melmaruvathur - 603 319.

CERTIFICATE

This is to certify that the thesis entitled **"METHOD DEVELOPMENT AND VALIDATION OF NEWER ANALYTICAL TECHNIQUES"** submitted to The TamilNadu. Dr. M.G.R. Medical University, Guindy, Chennai-600032, TamilNadu, India as a partial requirement for the award of Degree of Doctor of Philosophy (Faculty of Pharmacy) is a record of research work done by **Mrs. G.ABIRAMI, M. Pharm.,** during 2008 to 2013, under my guidance and supervision at Adhiparasakthi College of Pharmacy, Melmaruvathur, TamilNadu, India and that the thesis has not formed the basis for the award of any other degree, diploma, associateship, fellowship or any other similar title to the candidate and the thesis represents independent work of the candidate.

Place: Melmaruvathur
Date:

Prof. Dr. T. VETRICHELVAN, M.Pharm., Ph.D.,
Principal,
Adhiparasakthi College of Pharmacy,
Melmaruvathur - 603 319.

ACKNOWLEDGEMENT

It gives me immense pleasure to acknowledge, the help rendered to me by a host of a people, to whom I owe gratitude for successful completion of Ph.D. First and foremost, I wish to express my deep sense of gratitude to his Holiness **ARULTHIRU AMMA,** President, ACMEC Trust, and Melmaruvathur for his ever growing blessings in each step of the study.

I am grateful to **THIRUMATHI LAKSHMI BANGARU ADIGALAR,** Vice President, ACMEC Trust, Melmaruvathur for having given me an opportunity and encouragement all the way in completing the study.

The research work embodied in dissertation has been carried out under supervision of my esteemed and most respected guide **Prof. (Dr.) T. VETRICHELVAN, M.Pharm., Ph.D.,** Principal and Head, Department of Pharmaceutical Analysis, Adhiparasakthi College of Pharmacy, my greatest debt of gratitude is to him for their continuous encouragement, valuable suggestions, dynamic guidance, eve readiness to elucidate problems a source of inspiration and constant motivation throughout the dissertation work.

I express my deep sense of gratitude to respected **Prof. (Dr.) P.Perumal, M.Pharm., Ph.D.,** and **Prof. (Dr.) G.Krishnamoorthy M.Pharm., Ph.D.,** Doctoral committee members for their support for the research work.

I conceitedly take the dispensation to present my special wisdom of thanks to **Dr. (Mrs.) D. NAGAVALLI, M. Pharm., Ph.D.,** Professor, **Mr. K. ANANDAKUMAR, M. Pharm.,** Associate professor and **Ms. R.RADHA, M. Pharm.,** Assistant professor other faculty members of Adhiparasakthi college of

Pharmacy, Melmaruvathur, for their valuable help and guidance during the course of my research work.

I acknowledge the help and support rendered by our laboratory staff members **Mrs. S. KARPAGAVALLI, D.Pharm.. Mr. M.GOMATHISHANKAR, D.Pharm., Mrs. S.SHOBANA B.Sc.,** throughout my project work.

I am indeed very much thankful to the librarian **Mr. M. SURESH, M.L.I.S.,** Adhiparasakthi College of Pharmacy, for providing all reference books and journals for the completion of this project.

A word of thanks to office staffs **Mr. S.ELUMALAI, Mr. M. KARTHIKEYAN** and other members of our college for providing all the help when required.

I wish to regard my heartfelt thanks to **Mr. M.THIRUGNAM B.Sc., Senior Analyst, A.M.RAVISANKAR B.Sc.,** Senior Analyst, **Pharma Analytical Lab, at** Pondicherry to carry out the project work.

I wish to thanks **Mrs. NIRMALA M.Pharm.,** Lecturer, Vel's University Pallavaram to carry out the project work

I express my deep love and sincere sense of gratitude to my father **Mr. R.GANESAN** my beloved mother **Mrs. G.THAILNAYAKI,** my Husband **Mr. N.S.ASHOK KUMAR** for their support, guidance, inspiration and constant prayers for my successful endeavors.

Above all I dedicate myself and my work to **Almighty**, who is the source of knowledge and for showering all his blessings and grace upon me.

ABIRAMI.G

DEDICATED

TO MY

BELOVED PARENTS

CONTENT

\

LIST OF FIGURES

LIST OF TABLES

SYMBOLS AND ABBREVIATIONS

ICH	-	International Conference on Harmonization
λ	-	Lambda
SD	-	Standard Deviation
SE	-	Standard Error
UV-VIS	-	Ultraviolet - Visible
USP	-	United States Pharmacopoeia
IP	-	Indian Pharmacopoeia
BP	-	British Pharmacopoeia
IR	-	Infra Red
°C	-	Degree Celsius
LOD	-	Limit of Detection
LOQ	-	Limit of Quantitation
µg/ ml	-	Microgram per Millilitre
mg/ tab	-	Milligram per Tablet
ml	-	Millilitre
MM	-	Milli Mole
nm	-	Nanometre
pH	-	Negative Logarithm of Hydrogen Ion Concentration
%	-	Percentage
% RSD	-	Percentage Relative Standard Deviation

RP - HPLC	-	Reverse Phase -High Performance Liquid Chromatography
HPTLC	-	High Performance Thin Layer Chromatography
TPE	-	Tolperisone Hydrochloride
PCL	-	Paracetamol
SIM	-	Simvastatin
SITA	-	Sitagliptin Phosphate
THI	-	Thiocolchicoside
KET	-	Ketoprofen
DES	-	Desloratadine
AMB	-	Ambroxol Hydrochloride
DOX	-	Doxofylline
SAL	-	Salbutamol Sulphate
Gms	-	Grams
µl	-	Micro litre
Rpm	-	Rotation per minute
µ	-	Micron
V/v/v/v	-	Volume/Volume/Volume/Volume
min	-	Minute
ml/ min	-	Millilitre/minute
ng/ µl	-	Nanogram/ micro litre
hυ	-	Planck's constant

LC-MS	-	Liquid Chromatography Mass Spectrometry
GC-MS	-	Gas Chromatography Mass Spectrometry
GC	-	Gas Chromatography
CRF	-	Chromatographic Response Factor
USFDA	-	United States Food and Drug Administration
WHO	-	World Health Organization GLP
GMP	-	Good Laboratory Practice
S/N	-	Signal to Noise ratio
LDP	-	Low Density Lipoprotein
HMG – COA	-	Hydroxy Methyl Glutaryl – Co-Enzyme
CYP	-	Cytochrome Phosphate
COX	-	Cyclo Oxygenase
PGH_2	-	Prostaglandin Hydroxy Synthase
ILC	-	Inverse Least Square
PCR	-	Principle Component Regression
PLS	-	Partial Least Square
ODS	-	Octa Decyl Silane
AR	-	Analytical Reagent
NaI	-	Sodium Iodide
DPP-4	-	Dipeptidyl peptidase

INTRODUCTION

1. INTRODUCTION

Pharmaceutical products formulated with more than one drug, typically referred to as combination products, are intended to meet previously unmet patients need by combining the therapeutic effects of two or more drugs in one product. These combination products can present daunting challenges to the analytical chemist responsible for the development and validation of analytical methods.

The number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing one. Very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities (resulting in their withdrawal from the market), development of patient resistance and introduction of better drugs by competitors. Under these conditions, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs.

1.1. Basic criteria for new method development of drug analysis: [1]

- The drug or drug combination may not be official in any pharmacopoeias,
- A proper analytical procedure for the drug may not be available in the literature due to patent regulations,
- Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients,
- Analytical methods for the quantitation of the drug in biological fluids may not be available,

- Analytical methods for a drug in combination with other drugs may not be available,
- The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable.

1.2. INTRODUCTION TO SPECTROPHOTOMETRIC METHODS OF ANALYSIS FOR DRUGS IN COMBINATION [2]

Simultaneous estimation of drug combination is generally done by separation using chromatographic methods like HPLC, GC and HPTLC etc. These methods are accurate and precise with good reproducibility, but the cost of analysis is quite high owing to expensive instrumentation, reagent and expertise. Hence it is worthwhile to develop simpler and cost effective method for simultaneous estimation of drugs for routine analysis of formulation. Spectrophotometric analysis fulfils such requirement where the simultaneous estimation of the drug combination can be done with similar effectiveness as that of chromatographic methods.

A number of modifications to the simple spectrophotometric procedure are available to the analyst, which may eliminate certain sources of interference and permit the accurate determination of all of the absorbing components. Each modification of the basic procedure may be applied if certain criteria are satisfied.

The basis of all the spectrophotometric techniques for multicomponent samples is the property that at all wavelengths:

- the absorbance of a solution is the sum of absorbance of the individual components or

- 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 cell.

There are various spectrophotometric methods are available which can be used for the analysis of a combination samples. Following methods can be used

- Simultaneous equation method
- Derivative spectrophotometric method
- Absorbance ratio method (Q-Absorbance method)
- Difference spectrophotometry
- Solvent extraction method

Simultaneous Equation Method [2]

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

The in formations required are:

- the absorptivities of X at λ_1 and λ_2, a_{x1} and a_{x2} respectively
- the absorptivities of Y at λ_1 and λ_2, a_{y1} and a_{y2} respectively
- The absorbance of the diluted sample at λ_1 and λ_2, A_1 and A_2 respectively.

Therefore

$C_x = (A_1 a_{y2} - A_2 a_{y1}) / (ax_1 a_{y2} - a_{x2} a_{y1})$ …… (1)

$C_y = (a_{x1} A_2 - a_{x2} A_1) / (ax_1 a_{y2} - a_{x2} a_{y1})$ …….. (2)

Q - Absorbance Method (Absorbance Ratio Method) [2]

Q - Absorbance method depends on the property that, for a substance which obeys Beer's law at all wavelength, the ratio of absorbances at any two wavelengths is a constant value independent of concentration or path length. For example, two different dilution of the same substance give the same absorbance ratio A_1/A_2. In the USP, this ratio is referred to as Q value.

In the quantitative assay of two components in a mixture by the absorbance ratio method, absorbances are measured at two wavelengths. One being the λ max of one of the component (λ_2) and the other being a wavelength of equal absorptivities of the two components i.e. an isoabsorptive point[8].

Let $Q_X = a_{x2} / a_{x1,}$ $Q_Y = a_{y2}/ a_{y1,}$ and $Q_M = A_2 / A_1$

$$Q_M = F_x (Q_X - Q_Y) + Q_Y$$

$$F_x = (Q_M - Q_Y) / (Q_X - Q_Y)$$

Derivative Spectroscopy [2]

For the purpose of spectral analysis in order to relate chemical structure to electronic transitions, and for analytical situations in which mixture contribute interfering absorption, a method of manipulating the spectral data is called derivative spectroscopy. Derivative spectrophotometry involves the conversions of a normal spectrum to its first, second or higher derivative spectrum. In the context of derivative spectrophotometry, the normal absorption spectrum is referred to as the fundamental, zero order, or D^0 spectrum

Solvent Extraction Method [2]

In solvent extraction method quantitation of individual drugs in combinations has been performed by separation of individual drugs based on their selective solubility followed by spectrophotometric measurement[17]

1.3. INTRODUCTION TO HPLC METHODS OF ANALYSIS FOR DRUGS IN COMBINATION [3 – 5]

Most of the drugs in multi component dosage forms can be analyzed by HPLC method because of the several advantages like rapidity, specificity, accuracy, precision and ease of automation in this method. HPLC method eliminates tedious extraction and isolation procedures. Some of the advantages are:

- Speed (analysis can be accomplished in 20 minutes or less),
- Greater sensitivity (various detectors can be employed),
- Improved resolution (wide variety of stationary phases),
- Reusable columns (expensive columns but can be used for many analysis),
- Ideal for the substances of low volatility,
- Easy sample recovery, handling and maintenance,
- Instrumentation tends itself to automation and quantitation (less time and less labour),
- Precise and reproducible,
- Calculations are done by integrator itself,

Suitable for preparative liquid chromatography on a much larger scale.

1.3.1. System Suitability Tests for Chromatographic Methods [6, 7, 9]

System suitability is the checking of a system to ensure system performance before or during the analysis of unknowns. Parameters such as plate count, tailing factors, resolution and reproducibility (% RSD retention time and area for six repetitions) are determined and compared against the specifications set for the method. These parameters are measured during the analysis of a system suitability "sample" that is a mixture of main components and expected by-products. Lists of the terms to be measured and their recommended limits obtained from the analysis of the system suitability sample are given below.

System Suitability Parameters and Recommendations

Parameter	**Recommendation**
Capacity Factor (k')	the peak should be well-resolved from other peaks and the void volume, generally k'>2.0
Repeatability	RSD </= 1% for N >/= 5 is desirable.
Relative retention	not essential as long as the resolution is stated.
Resolution (R_s)	R_s of > 2 between the peak of interest and the closest eluting potential interferent (impurity, excipient, degradation product, internal standard, etc.
Tailing Factor (T)	T of </= 2
Theoretical Plates (N)	In general should be > 2000

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 (α)

The selectivity (or separation factor)α, 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.

1.4. INTRODUCTION TO HPTLC METHODS OF ANALYSIS FOR DRUGS IN COMBINATION [2, 7]

HPTLC (High Performance Thin Layer Chromatography) is a well known and versatile separation method which shows a lot of advantages in comparison to other separation techniques.

Layer of Sorbent	100µm
Efficiency	High due to smaller particle size generated
Separations	3 - 5 cm
Analysis Time	Shorter migration distance and the analysis time is greatly reduced
Solid support	Wide choice of stationary phases like silica gel for normal phase and C8 , C18 for reversed phase modes
Development chamber	New type that require less amount of mobile phase
Sample spotting	Auto sampler
Scanning	Use of UV/ Visible/ Fluorescence scanner scans the entire chromatogram qualitatively and quantitatively and the scanner is an advanced type of densitometer

1.4.1 Features of HPTLC

1. Simultaneous processing of sample and standard - better analytical precision and accuracy less need for Internal Standard
2. Several analysts work simultaneously
3. Lower analysis time and less cost per analysis
4. Low maintenance cost
5. Simple sample preparation - handle samples of divergent nature
6. No prior treatment for solvents like filtration and degassing
7. Low mobile phase consumption per sample
8. No interference from previous analysis - fresh stationary and mobile phases for each analysis - no contamination
9. Visual detection possible - open system
10. Non UV absorbing compounds detected by post-chromatographic derivatization

1.4.2. Steps involved in HPTLC

1. Selection of chromatographic layer
2. Sample and standard preparation
3. Layer pre-washing
4. Layer pre-conditioning
5. Application of sample and standard
6. Chromatographic development
7. Detection of spots
8. Scanning
9. Documentation of chromatic plate

1.4.3. Selection of chromatographic layer

- Precoated plates - different support materials - different Sorbents available

- 80% of analysis - silica gel GF · Basic substances, alkaloids and steroids Aluminium oxide

- Amino acids, dipeptides, sugars and alkaloids - cellulose

- Non-polar substances, fatty acids, carotenoids, cholesterol - RP2, RP8 and RP18

- Preservatives, barbiturates, analgesic and phenothiazines - Hybrid plates -RPWF254s

1.4.4. Sample and Standard Preparation

- To avoid interference from impurities and water vapours.

- Low signal to noise ratio - Straight base line- Improvement of LOD

- Solvents used are Methanol, Chloroform: Methanol (1:1), Ethyl acetate: Methanol (1:1),

- -- Chloroform: Methanol: Ammonia (90:!0:1), Methylene chloride : Methanol (1:1),

1% Ammonia or 1% Acetic acid

- Dry the plates and store in dust free atmosphere

1.4.5. Activation of pre-coated plates

- Freshly open box of plates do not require activation
- Plates exposed to high humidity or kept on hand for long time to be activated
- By placing in an oven at 110-120ºc for 30 minutes prior to spotting
- Aluminium sheets should be kept in between two glass plates and placing in oven at 110-120ºc for 15 minutes.

1.4.6. Application of sample and standard

- Usual concentration range is 0.1-1µg / µl
- Above this causes poor separation
- Linomat IV (automatic applicator) - nitrogen gas sprays sample and standard from syringe on TLC plates as bands
- Band wise application - better separation - high response to densitometer

Selection of mobile phase

Trial and error

one's own experience and Literature

Normal phase

Stationary phase is polar

Mobile phase is non polar-Non-polar compounds eluted first because of lower affinity with stationary phase

Polar compounds retained because of higher affinity with the stationary phase

Reversed phase

Stationary phase is non polar

Mobile phase is polar

Polar compounds eluted first because of lower affinity with stationary phase

Non-Polar compounds retained because of higher affinity with the stationary phase- 3 - 4 component mobile phase should be avoided

Multi component mobile phase once used not recommended for further use and solvent composition is expressed by volumes (v/v) and sum of volumes is usually 100

Twin trough chambers are used only 10 -15 ml of mobile phase is required Components of mobile phase should be mixed introduced into the twin - trough chamber

Pre- conditioning (Chamber saturation)

- Un- saturated chamber causes high Rf values

- Saturated chamber by lining with filter paper for 30 minutes prior to development - uniform distribution of solvent vapours - less solvent for the sample to travel - lower Rf values.

Chromatographic development and drying

After development, remove the plate and mobile phase is removed from the plate - to avoid contamination of lab atmosphere

Dry in vacuum desiccators - avoid hair drier - essential oil components may evaporate

Detection and visualization

Detection under UV light is first choice - non destructive

Spots of fluorescent compounds can be seen at 254 nm (short wave length) or at 366 nm (long wave length)

Spots of non fluorescent compounds can be seen - fluorescent stationary phase is used - silica gel GF

Non UV absorbing compounds like ethambutol, dicylomine etc - dipping the plates in 0.1% iodine solution

When individual component does not respond to UV - derivatisation required for detection

Quantification

- Sample and standard should be chromatographed on same plate

- After development chromatogram is scanned
- Camag TLC scanners III scan the chromatogram in reflectance or in transmittance mode by absorbance or by fluorescent mode
- scanning speed is selectable up to 100 mm/s - spectra recording is fast
- 36 tracks with up to 100 peak windows can be evaluated
- Calibration of single and multiple levels with linear or non-linear regressions are possible
- When target values are to be verified such as stability testing and

- Dissolution profile single level calibration is suitable
- Statistics such as RSD or CI report automatically
- Concentration of analyte in the sample is calculated by considering the sample

Initially taken and dilution factors.

1.5. VALIDATION [8, 12]

The word “Validation” means “Assessment” of validity or action of proving effectiveness.

Validation as defined by different agencies

USFDA - According to this “validation” is the process of establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes.

WHO - Defines validation as an action of providing any procedure process equipment material, activity or system actually leads to the expected results.

EUROPEON COMMITTEE - Defines validation as an action of providing in a accordance with the principles of GMP that any procedure, process material and activity or system actually leads to expected result.

1.5.1 Method Validation [8]

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.

Analytical methods need to be validated or revalidated

- before their introduction into routine use;
- whenever the conditions change for which the method has been validated (e.g., an instrument with different characteristics or samples with a different matrix); and

- Whenever the method is changed and the change is outside the original scope of the method.

The various validation parameters are:

  - Accuracy,
  - Precision (repeatability and reproducibility),
  - Linearity and Range,
  - Limit of detection (LOD)/ Limit of quantitation (LOQ),
  - Selectivity/ Specificity,
  - Robustness/ Ruggedness and
  - Stability and System suitability studies.

Advantages of Analytical method Validation:-

- The biggest advantage of method validation is that it builds a degree of confidence, not only for the developer but also to the user.
- Although the validation exercise may appear costly and time consuming, it results inexpensive, eliminates frustrating repetitions and leads to better time management in the end.
- Minor changes in the conditions such as reagent supplier or grade, analytical setup are unavoidable due to obvious reasons but the method validation absorbs the shock of such conditions and pays for more than invested on the process.

Key parameters of the Analytical method validation:-[19, 20]

It is important for one to understand the parameters or characteristics involved in the validation process. The various Performance parameters, which are addressed in a validation exercise, are grouped as follows.

(1) Accuracy: -

The accuracy of an analytical method may be defined as the closeness of the test results obtained by the method to the true value. It is the measure of the exactness of the analytical method developed. Accuracy may often express as percent recovery by the assay of a known amount of analyte added.

Accuracy may be determined by applying the method to samples or mixtures of excipients to which known amount of analyte have been added both above and below the normal levels expected in the samples. Accuracy is then calculated from the test results as the percentage of the analyte recovered by the assay.

(2) Precision: -

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. This is usually expressed as the standard deviation or the relative standard deviation (coefficient of variation). Precision is a measure of the degree of reproducibility or of the repeatability of the analytical method under normal operating circumstances.

Repeatability involves analysis of replicates by the analyst using the same equipment and method and conducting the precision study over short period of time while reproducibility involves precision study at

- Different Occasions,
- Different Laboratories,
- Different Batch of Reagent,
- Different Analysts,
- Different Equipments.

Determination of Repeatability*:* - Repeatability can be defined as the precision of the procedure when repeated by same analyst under the same operating conditions (same reagents, equipments, settings and laboratory) over a short interval of time.

Determination of reproducibility: - Reproducibility means the precision of the procedure when it is carried out under different conditions-usually in different laboratories-on separate, putatively identical samples taken from the same homogenous batch of material. Comparisons of results obtained by different analysts, by the use of different equipments, or by carrying out the analysis at different times can also provide valuable information.

(3) Linearity and range:-

The linearity of an analytical method is its ability to elicit test results that are directly (or by a well defined mathematical transformation) proportional to the analyte concentration in samples within a given range. Linearity usually expressed in terms of the variance around the slope of regression line calculated according to an established mathematical relationship from test results obtained by the analysis of samples with varying concentrations of analyte.

The linear range of detectability that obeys Beer's law is dependent on the compound analyzed and the detector used.

Data is processed by linear least square regression declaring the regression co-efficient and b of the linear equation y = ax + b together with the correlation coefficient of determination r. For the method to be linear the r value should be close to1.

The range of an analytical method is the interval between the upper and lower levels of the analyte (including these levels) that have been demonstrated to be determined with precision, accuracy and linearity using the method as written.

(4) Limit of Detection and limit of Quantitation:-

Limit of detection: - The limit of detection is the parameter of limit tests. It is the lowest level of analyte that can be detected, but not necessarily determined in a quantitative fashion, using a specific method under the required experimental conditions. The limit test thus merely substantiates that the analyte concentration is above or below a certain level.

The determination of the limit of detection of instrumental procedures is carried out by determining the signal-to-noise ratio by comparing test results from the samples with known concentration of analyte with those of blank samples and establishing the minimum level at which the analyte can be reliably detected. A signal-to-noise ratio of 2:1 or 3:1 is generally accepted.

Limit of quantitation*:* - Limit of quantitation is a parameter of quantitative assays for low levels of compounds in sample matrices such as impurities in bulk drugs and degradation products in finished pharmaceuticals. The limit of quantitation is the lowest concentration of analyte in a sample that may be determined with acceptable accuracy and precision when the required procedure is applied.

(5) Selectivity and Specificity:-

The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix.

Selectivity may be expressed in terms of the bias of the assay results obtained when the procedure is applied to the analyte in the presence of expected levels of other components, compared the results obtained when the procedure is applied to the analyte in the presence of expected levels of other components, compared to the results obtained on the same analyte without added substances.

6) Robustness and Ruggedness:-

Robustness: - The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage. The determination of robustness requires that methods characteristic are assessed when one or more operating parameter varied.

Ruggedness:- The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different laboratories, different analysts, using operational and environmental conditions that may differ but are still within the specified parameters of the assay. The testing of ruggedness is normally suggested when the method is to be used in more than one laboratory. Ruggedness is normally expressed as the lack of the influence on the test results of operational and environmental variables of the analytical method.

For the determination of ruggedness, the degree of reproducibility of test result is determined as function of the assay variable. This reproducibility may be compared to the

precision of the assay under normal condition to obtain a measure of the ruggedness of the analytical method.

(7) Stability and System suitability tests:-

Stability of the sample, standard and reagents is required for a reasonable time to generate reproducible and reliable results. For example, 24 hour stability is desired for solutions and reagents that need to be prepared for each analysis.

System suitability test provide the added assurance that on a specific occasion the method is giving, accurate and precise results. System suitability test are run every time a method is used either before or during analysis.

The results of each system suitability test are compared with defined acceptance criteria and if they pass, the method is deemed satisfactory on that occasion. The nature of the test and the acceptance criteria will be based upon data generated during method development optimization and validation experiments**.**

1.6. BASIC STATISTICAL PARAMETERS [17]

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

1.6.1 Linear Regression

Linear regression is 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 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.

$$m = \frac{N\sum xy - (\sum x)(\sum y)}{N\sum x^2 - (\sum x)^2} \quad \text{and} \quad c = \frac{(\sum y)(\sum x^2) - (\sum x)(\sum xy)}{N\sum x^2 - (\sum x)^2}$$

1.6.2 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.

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

Where n is the number of data points.

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

1.63 Standard Deviation (SD)

It is commonly used in statistics as a measure of precision statistics as a measure of precision and is more meaningful than is the average deviation. It may be thought of as a root-mean-square deviation of values from their average and is expressed mathematically as

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

Where,

S is standard deviation.

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

Σ = sum

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

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

N = Number of observations.

1.6.4 Percentage Relative Standard Deviation (%RSD)

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

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

Where,

SD = the standard deviation,

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

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

1.6.5 Standard Error of Mean (SE)

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

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

Where,

SD = Standard deviation.

n = number of observation

1.6.6 Confidence Interval (CI)

A confidence interval gives an estimated range of values which is likely to include a unknown population parameter, the estimated range being calculated from a given set of

sample data. A confidence interval with a particular confidence level (95% selected by the user) is intended to give the assurance that, if the statistical model is correct then the interval could deliver the true value.

Confidence interval for a normal population, $\bar{Y} \pm \frac{Z_{\alpha/2}\ \sigma}{\sqrt{N}}$

Where $\bar{Y}$ = Sample mean

$Z_{\alpha/2}$ = upper $\alpha/2$ critical value of standard normal distribution

N = Size of sample

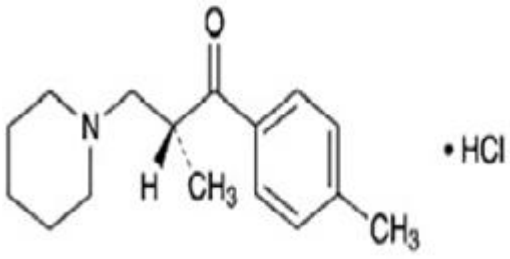
σ = Standard deviation

LITERATURE REVIEW

2.1 DRUG PROFILE

2.1.1 TOLPERISONE HYDROCHLORIDE [20, 21]

Molecular structure:



Chemical name:

2-methyl-1-(4-methylphenyl)-3-(1-piperidyl) propan-1-one

Molecular Formula:

$C_{16}H_{23}NO$

Molecular Weight:

281.81 g/mol.

Category:

Anti-spasmodic

Storage:

Store it at room temperature.

Description:

White amphorous powder.

Solubility: Freely soluble in water.

Melting Point:

Standard value	Observed value*
181-183℃.	182℃.

***Average of six determinations**

Pharmacological Parameters:

Side Effects: Adverse effects of Tolperisone includes

In hypersensitivity: muscle weakness, headache, arterial hypotension, nausea, vomiting, dyspepsia.

Skin allergic reactions: skin rash, hives, Quincke's edema, anaphylactic shock.

Contraindications:

Not to be used during Myasthenia, Children under 1 years of age, Pregnancy, Breastfeeding.

Interactions:

Tolperisone enhance the effects of other neuromuscular blocking agents.

Clinical Pharmacology:

Mechanism of action: Tolperisone suppressed the spinal monosynaptic reflex transmission in *vivo* as well as in *vitro* by both presynaptic and postsynaptic mechanisms. In general, Tolperisone was more potent inhibitor of the spinal reflexes, showed a relatively stronger depressant effect on electrical excitability of motoneurones, and on the A- fibre mediated afferent nerve conduction. On the other hand, both compounds equally depressed conduction in C-fibres. The ionic mechanisms underlying the effects were further analyzed by whole cell patch - clamp studies on dorsal root ganglion (DRG) cells isolated from newborn rats.

Dosage & Administration: It comes as a tablet, and taken with food.

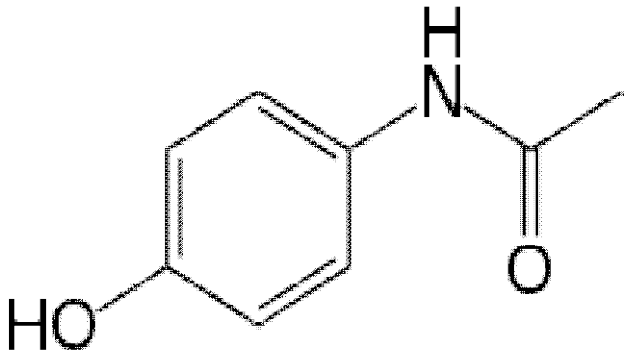
Adult: The recommended dose is 50-150 mg 3 times /day.

Pharmacokinetics:

Drugs are metabolized by the body in much the same way as food, herbals, and environmental pollutants; they are broken down by liver and gut enzymes or other *mechanisms* so they can be absorbed and eliminated in the bile and urine. Enzymes are available to metabolize specific substances- a medication is referred to as a *substrate* of the enzyme that can metabolize it.

2.1.2 PARACETAMOL[10, 19]

Molecular structure:



Chemical Name:

N- (4-hydroxy phenyl) ethanamide.

N- (4-hydroxy phenyl) acetamide.

Molecular Formula:

$C_8H_9NO_2$

Molecular Weight:

151.17g/mol

Dose: 0.5 to 1g up to 4g daily in divided doses

Description:

White crystalline powder, odourless, taste and slightly bitter.

Solubility:

Sparingly soluble in water, freely in alcohol, soluble in acetone and in solution of sodium hydroxide.

Assay: I.P 1996

Dissolved 0.3g in a mixture of 10 ml water and 30 ml of 2N sulphuric acid. Boiled under reflux for 1 hr, and diluted to 100 ml with water. To 20 ml of the solution added 40 ml of water, 40g of ice, 15 ml of 2N Hcl, and 0.1 ml ferroin sulphate solution and titrated with 0.1M Cerric ammonium sulphate until a yellow colour was obtained. Repeated the procedure without sample being examined. Each ml of 0.1M Cerric Ammonium Sulphate is Equivalent to 0.00756g of $C_8H_9NO_2$

Melting Point:

Standard value	Observed value*
169℃.	168℃.

***Average of six determinations**

Storage Conditions:

Tablet, syrup, suspension: Store in a well closed container, Below 40℃. Protect from Sunlight and Moisture.

Adverse effects:

In recommended doses, the side effects of Paracetamol are mild to non-existent. Paracetamol has fewer adverse gastrointestinal effects. Prolonged daily use increases the

risk of upper gastrointestinal complications such as stomach bleeding, and may cause kidney or liver damage.

Chronic users of Paracetamol May have a higher risk of developing blood cancer.

Overdose

Main article: Paracetamol toxicity

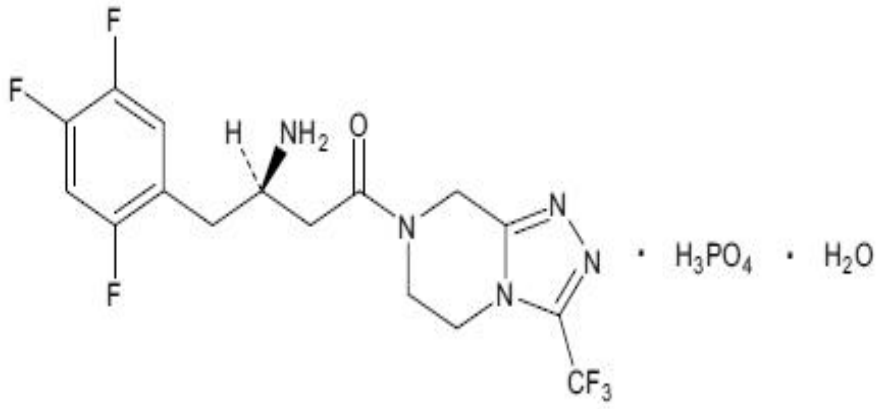
Paracetamol hepatotoxicity is, by far, the most common cause of acute liver failure in both the United States and the United Kingdom. Toxicity of paracetamol arises often due to its quinone metabolite. Paracetamol overdose results in more calls to poison control centers in the US than overdose of any other pharmacological substance. Signs and symptoms of Paracetamol toxicity may initially be absent or vague. Untreated overdose can lead to liver failure and death within days. Treatment is aimed at removing the Paracetamol From the body and replacing glutathione. Activated charcoal can be used to decrease absorption of Paracetamol if the patient presents for treatment soon after the overdose

Mechanism of Action:

The main mechanism proposed is the inhibition of Cyclooxygenase (COX), and recent findings suggest that it is highly selective for COX-2. While it has analgesic and antipyretic properties comparable to those of aspirin or other NSAIDs, its peripheral anti-inflammatory activity is usually limited by several factors, one of which is the high level of peroxides present in inflammatory lesions. However, in some circumstances, even peripheral anti-inflammatory activity comparable to NSAIDs can be observed.

2.1.3. SITAGLIPTIN PHOSPHATE [20, 24]

Chemical Structure:



Chemical Name:

(*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine.

Molecular Formula:

$C_{16}H_{15}F_6N_5O \cdot H_3PO_4.H_2O$

Molecular Weight:

523.32 g/mol.

Melting point:

Standard value	Observed value*
198℃- 202°C.	201℃.

*Mean of six observations

Description:

Sitagliptin Phosphate Monohydrate is a white to off-white, crystalline, non hygroscopic powder.

Solubility:

Soluble in methanol, N, N dimethyl for amide and water. Insoluble in isopropanol

Storage:

Store at 20°C

Adverse effects:

In clinical trials, adverse effects were as common with Sitagliptin (whether used alone or with metformin or Pioglitazone) as they were with placebo, except for extremely rare nausea and common cold-like symptoms. There is no significant difference in the occurrence of hypoglycaemia between placebo and Sitagliptin.

Side effects:

Signs of an allergic reaction: hives; difficulty breathing; swelling of face, lips, tongue, or throat.

Side effect such as: pancreatitis - severe pain in upper stomach spreading to back, nausea and vomiting, loss of appetite, fast heart rate; or fever, sore throat, and headache with a severe blistering, peeling, and red skin rash.

Less serious side effects may include: runny or stuffy nose, sore throat; headache; or nausea, stomach pain, diarrhoea.

Drug Interactions:

Sitagliptin is unlikely to interact with most other medications. In fact, there is only one known drug interaction with Sitagliptin. Taking Sitagliptin and Digoxin (Digitek®, Lanoxin®) together can slightly increase the level of Digoxin in blood.

Contraindications:

Acute Inflammation of the Pancreas, Recent Operation, Body Temperature More Than 101 Degrees F, Injury, Infection, Low Blood Sugar, Moderate to Severe Kidney Impairment.

Mechanism of action:

Sitagliptin works to competitively inhibit the enzyme dipeptidyl peptidase 4 (DPP-4). This enzyme breaks down the in cretins GLP-1 and GIP, gastrointestinal hormones released in response to a meal. By preventing GLP-1 and GIP inactivation, they are able to increase the secretion of insulin and suppress the release of glucagon by the pancreas. This drives blood glucose levels towards normal. As the blood glucose level approaches normal, the amounts of insulin released and glucagon suppressed diminishes, thus tending to prevent an "overshoot" and subsequent low blood sugar (hypoglycaemia) which is seen with some other oral hypoglycaemic agents.

Sitagliptin is recommended as a second line drug (in combination with other drugs) after the treatment based on a combination of diet and metformin fails.

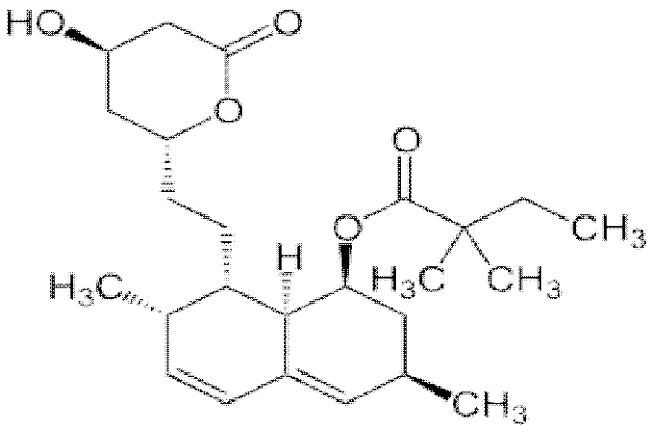
Pharmacokinetics:

Oral absorption of Sitagliptin is found to be 87%. Volume of distribution is found to be 198 litres and plasma protein binding is 38%. And metabolism is reported by

CYP3A4 and CYP2C8. Renal Excretion accounts for primarily active tubular secretion and plasma half life is 8-14 hour

2.1.4 SIMVASTATIN[20, 21]

Chemical Structure:



Chemical Name:

(1*S*, 3R, 7S, 8S, 8aR)-8-{2-[(2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2dimethylbutanoate.

Molecular Formula:

$C_{25}H_{38}O_5$

Molecular Weight:

418.566 g/mol

Melting Point:

Standard value	Observed value*
135℃- 138°C.	136℃.

***Average of six determinations**

Description:

White Amorphorous powder

Solubility:

Insoluble in water, soluble in Methanol

Storage:

Store below 40°C. Protect from Sunlight and Moisture.

Side Effects:

The severe or irreversible adverse effects of Simvastatin, which give rise to further complications, include acute renal failure.

The symptomatic adverse reactions produced by Simvastatin, can be treated symptomatically, these include Flatulence, Headache, Fatigue, Nausea, Diarrhoea, Constipation, Abdominal pain, Elevation of liver enzymes, Myopathy, Rhabdomyolysis, Muscle tenderness, Increased intracranial pressure, Hepatitis, Pancreatic.

Contraindications:

Simvastatin is contraindicated with pregnancy, breast feeding and liver disease. Pregnancy must be avoided while on Simvastatin due to potentially severe birth defects. Patients cannot breast feed while on Simvastatin due to potentially disrupting the infant's lipid metabolism. Simvastatin is also contraindicated with Amlodipine and should not exceed a dosage greater than 20mg/day when taken alongside Amlodipine.

Mechanism of Action:

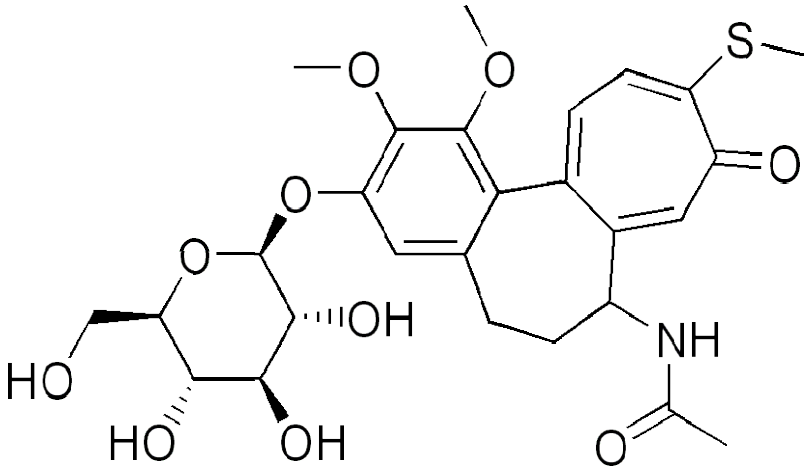
HMG CoA reductase inhibitors competitively inhibit the activity of HMG CoA reductase, the rate-limiting enzyme in cholesterol synthesis. Inhibition of this enzyme results in a transient, modest decrease in cellular cholesterol concentration. The decrease in cholesterol concentration activates a cellular signalling cascade culminating in the activation of sterol regulatory element binding protein (SREBP), a transcription factor that up-regulates expression of the gene encoding the LDL receptor. Increased LDL receptor expression causes increased uptake of plasma LDL, and consequently decreases plasma LDL-cholesterol concentration. Approximately 70% of LDL receptors are expressed by hepatocytes, with the remainder expressed by a variety of cell types in the body.

Pharmacokinetics:

Oral absorption of Simvastatin is found to be 42.5% ±42.5. Volume of distribution is found to be 98% and plasma protein binding is ~95%. Presystemic metabolism is noted to be 83% ±7 and metabolism is reported Hepatic. Renal Excretion accounts for 13% and plasma half life is 1.9 hr.

2.1.5. THIOCOLCHICOSIDE [21, 24]

Chemical Structure:



Molecular Formula:

$C_{27}H_{33}NO_{10}S$

Chemical name:

N-[(7*S*)-3-(beta-D-glucopyranosyloxy)-1,2-dimethoxy-10(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[a]heptalen-7-yl]acetamide

Molecular Weight:

563.618 g/mol

Melting Point:

Standard value	Observed average value*
208 ºC – 213ºC	211ºC

***Average of six observations**

Description:

Yellow crystalline powder

Solubility:

Soluble in water & slightly soluble in alcohol

Storage:

Store in controlled room temperature and Keep away from strong direct light

Dosage:

Oral

Muscle spasms

Adult: Initially, 16 mg daily.

Intramuscular

Muscle spasms

Adult: Up to 8 mg daily.

Category:

Muscle relaxant with anti-inflammatory and analgesic effects.

Mechanism:

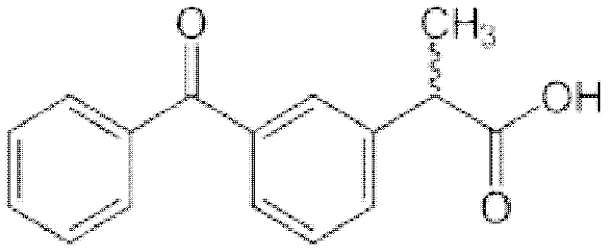
It acts as a competitive $GABA_A$ receptor antagonist and also glycine receptor antagonist with similar potency and nicotinic acetylcholine receptors to a much lesser extent. It has powerful convulsant activity and should not be used in seizure-prone individuals.

Side effects:

Side effect of skeletal muscle relaxants may include: sedation, drowsiness, blurred or double vision, constipation or diarrhoea, dizziness and drowsiness, nervousness and confusion, dry mouth, dyspepsia (chronic or recurrent pain in the upper abdomen, upper abdominal fullness, and feeling full earlier than expected when eating), fatigue, headache, heartburn, hiccups and nausea, insomnia, stomach cramps, trembling, vomiting, and weakness; and possible dependence following long-term use

2.1.6. KETOPROFEN[20.21]

Chemical Structure:



Chemical name:

(*RS*)-2-(3-benzoylphenyl) propanoic acid

Molecular formula:

$C_{16}H_{14}O_3$

Molecular Weight:

254.281 g/mol

Melting Point:

Standard value	**Observed average value***
94 °C – 95°C	**95°C**

***Average of six observations**

Description:

It is a white or off-white, odourless, non hygroscopic, fine to granular powder.

Solubility:

It is freely soluble in ethanol, chloroform, acetone, and ether and soluble in benzene and strong alkali, but practically insoluble in water at 20° C

Uses:

Ketoprofen is generally prescribed for arthritis-related inflammatory pains or severe toothaches that result in the inflammation of the gums.

Storage :

Store below 30°C

Mechanism of Action:

Ketoprofen exhibits anti-inflammatory, analgesic and antipyretic activities. It potently inhibits the enzyme cyclooxygenase resulting in prostaglandin synthesis inhibition. It also prevents formation of thromboxane A_2 by platelet aggregation.

Pharmacokinetics:

Absorption: Readily absorbed from the GI tract (oral); reduced absorption with food. Peak plasma concentrations after 0.5-2hr. Well absorbed (IM, rectal); minimal (topical). **Distribution:** Synovial fluid (substantial concentrations). Protein-binding: 99%. **Metabolism:** Hepatic via conjugation with glucuronic acid. **Excretion:** Urine (as glucuronide conjugates); 1.5- 4 hr (elimination half-life)

Adverse Drug Reactions:

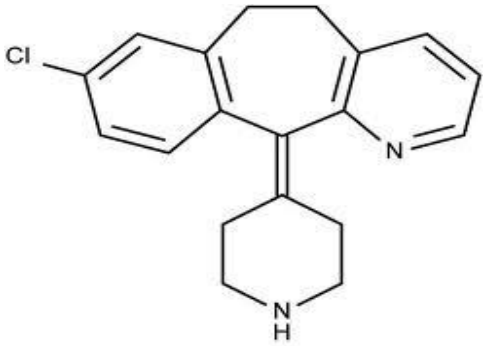
Acute interstitial nephritis, reversible decline in renal function; GI symptoms e.g. discomfort, nausea, diarrhoea; pain and tissue damage at inj site (IM). **Potentially Fatal:** Rarely, idiosyncrasy, anaphylaxis; very rarely GI haemorrhage

Contraindications

Acute peptic ulcer or dyspepsia.

2.1.7 DESLORATADINE [16, 20]

Chemical Structure:



Chemical Name:

4-(8-Chlor-5, 6-dihydro-11H-benzo [5, 6] cyclohepta [1, 2-b] pyrid-11-yliden) piper dine (IUPAC)

8-Chlor-11-(piperidin-4-yliden) -6, 11- dihydro-5H-benzo [5, 6] cyclohepta [1, 2-b] pyridine (IUPAC)

8-Chloro-6, 11-dihydro-11-(4-piperidylidene)-5H-benzo [5, 6] cyclohepta-[1, 2-b] pyridine (WHO)

Molecular formula:

C_{19}-H_{19}-Cl-N_2

Molecular Weight:

310 g/mole

Melting Point:

Standard value	**Observed average value***
150 ºC – 151°C	**151**°C

***Average of six observations**

Storage Conditions:

Store below 30°C.

Store at room temperature away from light and moisture.

Adverse effects:

Headache, fatigue, somnolence, dizziness; nausea, dyspepsia; xerostomia, dysmenorrhoeal; pharyngitis Nasal congestion is among the most bothersome of the symptoms of intermittent allergic rhinitis (IAR). Decongestants such as pseudoephedrine are often accompanied by adverse effects and should be avoided by patients with hypertension, arrhythmia, and other medical conditions. Most of the currently available antihistamines are ineffective for nasal congestion.

Dosage: Desloratadine, administered once daily at a dose of 5 mg, demonstrated significant improvement in nasal congestion/stuffiness at all time points assessed in the

study. This benefit was observed as early as the first patient evaluation on day 2 and continued throughout the 2 weeks of the study. Desloratadine is a new treatment option for patients with IAR and nasal congestion.

Overdose:

Desloratadine overdose symptoms may include:

- Excessive sleepiness
- Increased heart rate.

Mechanism of Action:

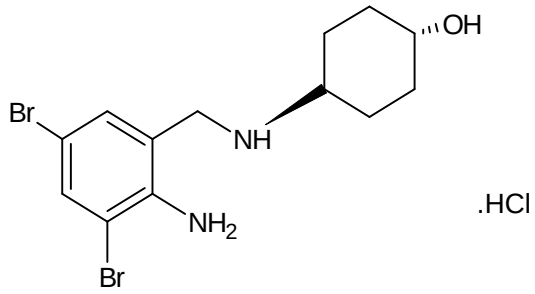
Desloratadine is a tricyclic antihistamine, which has a selective and peripheral H_1-antagonist action. It is an antagonist at histamine H1 receptors, and an antagonist at all subtypes of the muscarinic acetylcholine receptor. It has a long-lasting effect and in moderate and low doses, does not cause drowsiness because it does not readily enter the central nervous system. Unlike other antihistamines, desloratadine is also effective in relieving nasal congestion, particularly in patients with allergic rhinitis

Metabolism:

Desloratadine is a long-acting, tricyclic, non-sedating, selective peripheral histamine H1-receptor antagonist which inhibits the release of pro-inflammatory mediators from human mast cells and basophils.

2.1.8 AMBROXOL HYDROCHLORIDE [20, 24]

Chemical Structure



Chemical Name

Trans-4-(2-Amino-3, 5-dibrombenzylamino) - cyclohexanol hydrochloride.

Molecular formula:

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

Molecular weight:

414.6g/mol

Category:

Mucolytic agent; Expectorant

Description:

A white or yellowish crystalline powder

Solubility:

Sparingly soluble in water; Soluble in methanol and practically insoluble in methylene chloride

pH

A 1% solution in water has a pH of 4.5 to 6.

Standard:

Ambroxol hydrochloride contains not less than 99.0% and not more than 101.0% of $C_{13}H_{18}Br_2N_2O$, calculated on the dried basis

LOD

NMT 0.5%, determined on 1.0 gm by drying in an oven at 105°C

Assay:

Dissolve 0.3 gm in 70 ml of ethanol. Titrate with 0.1 M NaOH, determining the end point potentiometrically. Carry out blank. 1 ml of 0.1 M NaOH is equivalent to 0.04146 gm of Ambroxol hydrochloride.

Melting point:

Standard value	**Observed average value***
232 °C -234°C	**233°C**

***Average of six observations**

Storage:

1. Protect from light. Following reconstitution, aliquot and freeze at -20°C. This product is stable for 2 years as supplied

2. Stock solutions are stable for 4 months at -20°C

Indication:

It is primarily indicated in conditions like Bronchitis, Chronic bronchitis, Cystic fibrosis

Mode 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 defence mechanisms. It stimulates synthesis and release of surfactant by type II pneumocytes. Surfactants act 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.

Adverse drug reaction

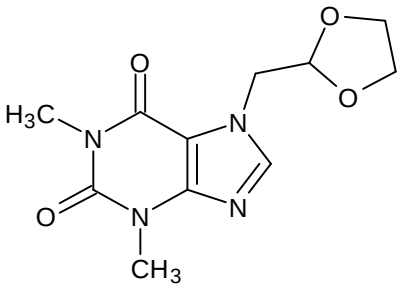
The symptomatic adverse reactions produced by Ambroxol Hcl are more or less tolerable and if they become severe, they can be tolerated symptomatically, these include Hypersensitivity reactions and Contact allergy.

Over dosage:

No symptoms of over dosage have been reported in man due to date. If they occur, symptomatic treatment should be provided.

2.1. 9 DOXOFYLLINE[20]

Chemical Structure



Chemical name

7-(1, 3-dioxolan-2-ylmethyl) - 1, 3-dimethylpurine-2,6-dione

Molecular formula

$C_{11}H_{14}N_4O_4$

Molecular weight

266.26 g/mol

Category

Anti-asthmatic

Description

White crystalline powder

Solubility:

Soluble in water, acetone, ethyl acetate, benzene, chloroform, dioxane, hot methanol and hot ethanol; practically insoluble in ethyl ether or petroleum ether.

Storage:

Store in a cool, dark and dry place

Melting point:

Standard value	Observed average value*
144 ºC – 145.5ºC	145ºC

***Average of six observations**

Indication:

Doxofylline is primarily indicated for Bronchial asthma, Bronchospasm and Chronic asthmatic bronchitis.

Mode of action:

Doxofylline is methyl xanthine derivatives and plays the direct role in relaxation of bronchial smooth muscle and thus acts as bronchodilator.

Doxofylline is the inhibitor of Phosphodiesterase and thus increases the intracellular level of cyclic- 3', 5'- adenosine monophosphate (cAMP) which produce bronchodilator and thus achieving suppression asthma role.

Pharmacokinetics:

Plasma protein binding is 48%. Renal excretion accounts for less than 4% and plasma half life is 7.42 hours.

Adverse Reaction:

Nausea, vomiting, epigastric pain, cephalalgia, irritability, insomnia, tachycardia, extra systole, tachypnea, hyperglycemia, albuminuria.

Contraindication:

Doxofylline is contraindicated in conditions like Acute Myocardial infarction, Hypersensitivity to xanthine derivatives.

Route of administration:

1. It is given by mouth in doses up to 1200 mg daily

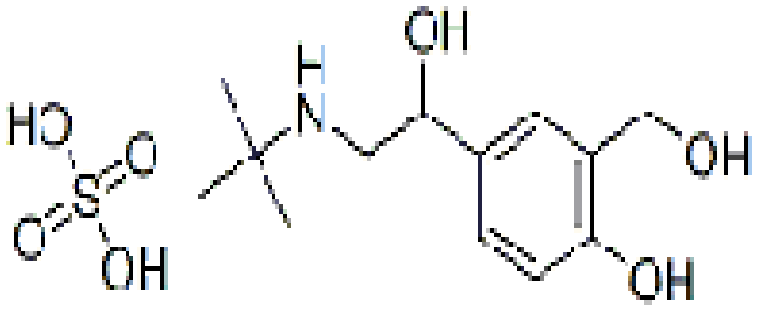
2. It may also be given by slow intravenous injection

Special Precaution:

Liver disease, Congestive Heart Failure, Chronic Obstructive Lung Disease, Concomitant Infection.

2.1.10 SALBUTAMOL SULPHATE [19, 20, 24]

Chemical Structure:



Chemical Name:

(RS)-4- [2-(tert- butyl amino) -1-hydroxyethyl]-2-(hydroxymethyl) phenol.

Molecular Formula:

$C_{13}H_{21}NO_3$

Molecular Weight:

239.311 g/mol.

Melting Point:

Standard value	Observed average value
157-158 ^{o}C	**156 ^{o}C**

Storage Conditions:

Store it at room temperature (30^{o}C).

Description:

White crystalline powder

Side Effects:

Central Nervous System - Nervousness, shakiness, dizziness, headache, over active and hoarseness.

Musculoskeletal - Muscle cramps.

Gastrointestinal - Nausea and increased or decreased appetite.

Respiratory - Nosebleed and difficulty in breathing.

Heart - Chest pain and irregular heart beat.

Miscellaneous - Fever, pale skin, rash, hives and itching.

Contraindicated in patients with high blood pressure during pregnancy, uterine Infection, miscarriage, heart disease, and hypersensitivity.

Mechanism of Action:

Salbutamol sulphate is a beta 2 adrenoceptor agonist. It binds therefore to Beta 2 receptors found particularly in the bronchioles of the respiratory system. In binding to these receptors it activates the Gs protein that the receptor is associated with and GDP is exchanged for GTP. This then activates the enzyme adenylate cyclase that coverts ATP into cAMP (a secondary messenger). Increased intracellular cyclic AMP (cAMP) increases the activity of cAMP dependent protein kinase A, which alters the phosphorylation of myosin and lowers intracellular calcium levels within the muscle. Lower levels of calcium cause relaxation of the smooth muscle and therefore bronchodilatation.

Dosage:

PO - The recommended dose is 2 to 4 mg.

IV/IM - 0.25 to 0.5mg.

Inhaler - 100 to 200 mg by inhalation.

Pharmacokinetic Data:

Metabolism - Hepatic

Half-life - 1.6 hours

Excretion - Renal.

Drug Interactions:

1. Administration of Ambroxol together with antibiotics (Amoxycillin, Cefuroxime, Erythromycin, and Doxycycline) leads to higher antibiotic concentration in the lung tissue.

2. No clinically relevant unfavourable interaction with other medications has been reported.

Contraindication:

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

2.2 REPORTED METHODS

Reported Methods for Tolperisone Hydrochloride:

1. Hariyani Kaushik P *et al.* [34] (**2012)** reported **"Spectrophotometric method for simultaneous estimation of Tolperisone hydrochloride and Diclofenac sodium in synthetic mixture."** Simultaneous equation method was employed for analysis at 255nm and 281nm were selected for the estimation of Tolperisone hydrochloride and Diclofenac sodium, respectively.
2. Monali Patel. *et al.* [35] **(2012)** reported "**Method Development and Statistical Validation of UV Spectrophotometric method for estimation of Tolperisone Hydrochloride and Paracetamol in Synthetic Mixture and Combined dosage Form."** The first UV spectrophotometric method was a determination using the simultaneous equation method at 242.5 nm and 260 nm. The second UV spectrophotometric method is the Q – analysis (absorption ratio) method, which involves the formation of absorbance equation at 254 nm (isoabsorptive point) and at 260 nm.
3. M. G. Patel *et al.* [36] **(2012)** reported **"The Simultaneous Estimation of Paracetamol and Tolperisone Hydrochloride in Tablet by UV Spectrophotometric Methods."** The first UV spectrophotometric method was a determination using the simultaneous equation method at 242.5 nm and 260 nm. The second UV spectrophotometric method is the Q – analysis (absorption ratio) method, which involves the formation of absorbance equation at 254 nm (iso absorptive point) and at 260 nm the maximum absorption of Tolperisone Hydrochloride.

4. Koladiya Bhavesh B. *et al.* [37] **(2012)** reported **"UV Spectrophotometric Method: A Quantitative Estimation of Tolperisone Hydrochloride in Bulk and Pharmaceutical Dosage Form."** The absorbance was measured at 260 nm using purified water as a solvent and the calibration curve was found to be linear in the concentration range of 3 -18μg/ml.
5. Carolin Nimila *et al.* [38] **(2011) r**eported **"Method development and statistical validation of UV spectrophotometric method for Tolperisone hydrochloride in bulk and tablet dosage form.''** A simple, novel, sensitive, and specific spectrophotometric method was developed and validated for the determination of Tolperisone Hydrochloride in bulk and its dosage form. The drug was estimated by using water as solvent for this study, which is determined by spectrophotometrically at 260 nm.
6. MM Sorathiya V *et al.* [39] **(2011)** reported **"Simultaneous Estimation of Paracetamol and Tolperisone Hydrochloride in Bulk and Combined Dosage Form by Derivative spectrophotometric method."**A simple, novel, sensitive, and specific spectrophotometric method was developed and validated for the determination of Paracetamol and Tolperisone Hydrochloride in bulk and its combined dosage form. First order derivative spectroscopy method is adopted to eliminate spectral interference. The method obeys Beer's Law in concentration ranges selected for evaluation. Paracetamol and Tolperisone hydrochloride have λ max at 248 nm and 255 nm respectively
7. Mandhanya Mayank, V *et al.* [40] **(2011)** reported "**Simultaneously Estimation of Paracetamol, Aceclofenac and Rabeprazole in Tablet Dosage Form Using UV Spectroscopy."** A simple, sensitive, reliable and rapid spectroscopic method has

been developed for the determination of Paracetamol, Aceclofenac and Rabeprazole in combined tablet dosage form.

8. Singh *et al.* [41] (**2011)** reported **"Validated RP - HPLC Method for the Simultaneous Estimation of Paracetamol and Naproxen in Tablet Formulation."** The proposed RP-HPLC method utilizes Eclipse XDB C_{18} column (150 ×4.6 mm i.d., 5 μm), optimum mobile phase consisted of gradient run of initial ratio of water (pH-2.5 adjusted with orthophosphoric acid: acetonitrile (87:13) with the effluent flow rate of 1.0 ml/min, and UV detection wavelength 263 nm.
9. Satyanarayana. P. V. V *et al.* [42] **(2011)** reported **"Simple validated isocratic RP – HPLC method for estimation of Tolperisone Hydrochloride in bulk and pharmaceutical dosage form."** The estimation was carried out on Inertsil ODS C-18, 5μm column having 250 x 4.6mm internal diameter column with a mixture of methanol: acetonitrile in the ratio of 90:10(v/v) as mobile phase. UV detection was performed at 232 nm.
10. P.Sai Praveen *et al.* [43] **(2011)** reported **"Spectrophotometric determination of Tolperisone Hydrochloride using 2, 4-dinitrophenylhydrazine reagent"** The proposed method was based on the interaction of the drug with 2, 4-dinitrophenylhydrazine in the presence of an acid catalyst, followed by treatment with a methanolic solution of potassium hydroxide; an intensely colored chromogen was formed that was measured in dim ethyl forma-mide as the diluting solvent at 520 nm.

Reported Methods for Paracetamol:

11. Buddha Ratna Shrestha *et al.* [44] (**2009) reported "Spectrophotometric Method for the Determination of Paracetamol."** Paracetamol with 1-napthol or resorcinol gave azodye and the concentration of Paracetamol was investigated spectrophotometrically. The azodyes formed with both 1-napthol and resorcinol as coupling agents follow Lambert Beer's law in the range of 0 to 10 μgmL^{-1} of Paracetamol.
12. Dhara J.Patel *et al.* [45] **(2010)** reported **"Simultaneous Determination of Paracetamol and Lornoxicam in Tablets by Thin Layer Chromatography Combined with Densitometry.''** The separation was carried out on Merck TLC aluminum sheets of silica gel 60F-254 using ethyl acetate: methanol: toluene: glacial acetic acid (7:2.5:1:0.5, v/v/v/v) as a mobile phase.
13. Patcharawee Nunthanavanit *et al.* [46] **(2010) reported "Simultaneous determination of Paracetamol and its main degradation product in generic Paracetamol tablets using reverse-phase HPLC."** The analytes were separated on a C_{18} Inertsil® ODS-3 column (250mm x 4.60 mm i.d., 5 μm particle size). A mobile phase, MeOH: 0.01M phosphate buffer pH5.0 (30:70 v/v) at flow rate of 1 ml/min was suitable for the separation and determination of Paracetamol and *p*-aminophenol. The UV detection was carried out at 243 nm
14. Godse VP *et al.* [47] **(2009) reported "Reverse Phase HPLC Method for Determination of Aceclofenac and Paracetamol in Tablet Dosage Form.''** A simple, rapid and selective HPLC method has been developed for quantitation of Aceclofenac and Paracetamol from bulk drug and pharmaceutical formulations using a mobile phase consisting mixture of methanol and water (70:30 v/v) at the flow rate of 1mL/min.

15. S. R. Pattan *et al.* [48] **(2009)** reported **"RP- HPLC Method for Simultaneous Estimation of Paracetamol and Etoricoxib from Bulk and Tablets."** The method was carried out on an inertsil ODS, 5μ, C8-3 column, with a mobile phase consisting of methanol: acetonitrile: phosphate buffer pH 3.5 (40:20:40 v/v) at a flow rate of 1.0 ml/min. Detection was carried out at 242. The retention time of Paracetamol and Etoricoxib were 3.27, 6.12 min. respectively.
16. C.Barbas *et al.* [49] **(2002)** reported **"Validation of a HPLC quantification of Acetaminophen, Phenylephrine and Chlorpheniramine in pharmaceutical formulations**: **capsules and sachets."** The selectivity of the method was also tested to be used if phenyl propanolamine hydrochloride were employed instead of phenylephederine. Final chromatographic conditions were a gradient elution, being solvent A: phosphate buffer 40 mM at pH 6.0 and solvent B: acetonitrile.
17. Janhavi R Rao *et al.* [50] **(2001)** reported **"**Development **and validation of HPLC method for Simultaneous quantitation of Paracetamol and Dexketoprofen trometamol in Bulk drug and formulation."** Thermo Hypersil ODS–C18 (250 mm × 4.6 mm, 5.0 μ) used as stationary phase and methanol: ammonium acetate buffer (65: 35 v/v) as mobile phase at a flow rate of 1.0 ml/min and the detection wavelength was 256 nm. The retention time for Paracetamol and Dexketoprofen was found to be 3.20 and 5.94 min, respectively.
18. M. Levent Altun et *al.* [51] **(2001)** reported **"HPLC Method for the Analysis of Paracetamol, Caffeine and Dipyrone.''** Paracetamol, Caffeine and Dipyrone were separated using a_-Bonda pack C_8 column by isocratic elution with a flow rate of 1.0 ml/min. The mobile phase composition was 0.01 M KH_2PO_4−-methanol- acetonitrile-isopropyl alcohol (420: 20: 30: 30) (v/v/v/v) and spectrophotometric detection was carried out at 215 nm.

19. Prasanna Reddy Battu *et al.* [52] **(2000)** reported **"RP-HPLC Method for Simultaneous Estimation of Paracetamol and Ibuprofen in Tablets.** Chromatographic separation achieved isocratic ally on a C_{18} column [Use Inertsil C18, 5m ,150 mm x 4.6 mm] utilizing a mobile phase of acetonitrile/phosphate buffer (60:40 v/v, pH 7.0) at a flow rate of 0.8 ml/min with UV detection at 260 nm.

Reported Methods for Sitagliptin Phosphate:

20. Safaa M Riad, *et.al.*[53] **(2012)** reported **"Spectrophotometric Determination of Sitagliptin and Metformin in their Pharmaceutical Formulation."** by using distilled water as a solvent and the first method was based on measuring the absorbance of at 268 nm in the range of 25-500 μg mL^{-1}. The second method was the isobestic point method. The total mixture concentration was calculated by measuring the absorbance at 257 nm.
21. T. Raja *et.al.* [54] **(2012)** reported "**Validated HPTLC Method For Simultaneous Estimation of Metformin Hydrochloride and Sitagliptin Phosphate in Bulk Drug and Formulation."** by using aluminium plates precoated with silica gel 60f 254 as the stationary phase and the solvent system consisted of acetone: methanol: toluene: formic acid (4:3:2:1 v/v/v/v) and scanned at 220 nm.
22. Jain Pritam, *et.al.*[55] **(2011)** reported **"Development and Validation of First Order Derivative UV- Spectrophotometric Method for Determination of Sitagliptin in Bulk and in Formulation."** By using methanol and water as a solvent.
23. T. Raja *et.al.*[56] **(2012)** reported **"Validated RP-HPLC Method For Simultaneous Estimation of Metformin Hydrochloride and Sitagliptin**

Phosphate in Bulk Drug and Pharmaceutical Formulation." by using mobile phase consisted of ethanol : acetonitrile: phosphate buffer in the ratio of 20:35:45 v/v/v (phosphate buffer P^H 8 was adjusted with sodium hydroxide) and detected at 254 nm.

24. Hitesh P. Inamdar *et.al.* [57] **(2012)** reported "**RP-HPLC Method for Simultaneous Determination of Metformin Hydrochloride, Rosiglitazone and Sitagliptin – application to commercially available drug products."** by using Water: ACN (70:30 %v/v) having p^H 3.0 as a solvent and the mobile phase at a flow rate of 1.5 ml min^{-1} consisted of 10 mm sodium hexane sulphonate monohydrate and 10 mm potassium dihydrogen phosphate buffer with acetonitrile and methanol in gradient ratio. The UV detection was carried out at 210 nm.
25. Sheetal Sharma *et.al.* [58] **(2012)** reported **"Development of UV - Spectrophotometry and RP-HPLC Method and its Validation for Simultaneous Estimation of Sitagliptin Phosphate and Simvastatin in marketed formulation."** The first method was based on spectrophotometric determination of two, using simultaneous equation method. It involves absorbance measurement at 267.0 nm (λ_{max} Sitagliptin phosphate) and 238.0 nm (λ_{max} Simvastatin) in methanol: water in a ratio of 90:10(v/v). The second method was done by using dihydrogen orthophosphate and acetonitrile. (50:50) as a mobile phase.
26. Swati Kupkar *et al.*[59] **(2012)** reported **"Simultaneous estimation of Sitagliptin and Metformin hydrochloride in bulk and dosage form by UV spectrophotometry.''** simultaneous determination of Sitagliptin and Metformin Hcl in bulk and dosage form by UV spectrophotometric method involves first

order derivative spectroscopy using 238.5 nm & 216.0 nm as zero crossing points for Sitagliptin and Metformin Hcl using 0.1 N NaOH was used as a solvent.

27. Dhiraj Kumar *et.al.* [60] (**2012**) reported **"Method Development and Estimation of Sitagliptin Phosphate in Bulk and Pharmaceutical Dosage Forms Using UV-Vis Spectrophotometer."** By using Distilled Water and Acetic Acid in Ratio of 80:20 and the λ max and the absorption maxima of the drug was found to be 268 nm.
28. Srinivasa rao Atla *et.al.*[61] **(2012)** reported **"Validated RP-HPLC Method for the Simultaneous Estimation of Sitagliptin and Simvastatin in Dosage Forms."** using acetonitrile: 0.1% orthophosphoric acid in water (70:30% v/v) as mobile Phase and the eluents were detected at 254 nm using UV detector.
29. Ankur Kothari *et.al.*[62] **(2012)** reported **"Development and Validation of Spectrophotometric Method for Simultaneous Estimation of Sitagliptin Phosphate and Simvastatin in Tablet Dosage Form."** by using methanol: water (90:10) and a simultaneous equation method was developed by measuring absorbance at 267.0 nm for Sitagliptin and 238.0 nm for Simvastatin .
30. Narendra nyola *et.al.*[63] **(2012)** reported **"Method Development of Simultaneous Estimation of Sitagliptin and Metformin Hydrochloride in Pure and Tablet Dosage form by UV-Vis Spectroscopy."** by using distilled water as a solvent and the maximum wavelength (λ max) of Metformin and Sitagliptin were found to be 231 nm and 267 nm respectively.
31. Amruta B. Loni *et al.* [64] **(2012)** reported **"Simultaneous UV Spectrophotometric Method for Estimation of Sitagliptin Phosphate and Metformin Hydrochloride in Bulk and Tablet Dosage Form."** Two simple, precise and economical UV methods have been developed for the simultaneous estimation of

Sitagliptin phosphate and Metformin hydrochloride in bulk and pharmaceutical dosage form. Method A is Absorbance maxima method, which is based on measurement of absorption at maximum wavelength of 266 nm and 232 nm for Sitagliptin phosphate and Metformin hydrochloride respectively. Method B is area under curve (AUC), in the wavelength range of 244-279 nm for Sitagliptin phosphate and 222-240 nm for Metformin hydrochloride.

32. N. Monila *et al.* [65] **(2012)** reported **"New Extractive Method Development of Sitagliptin Phosphate in API and its unit dosage forms by Spectrophotometry."** By using methanol as a solvent **t**wo proposed methods are based on complexation of the drug with bromo thymol blue & bromo cresol green), extracted with chloroform, showing absorbance maxima at 412 nm and 419 nm respectively.
33. Narendra Nyola et *al.* [66]**(2012)** reported **"Analytical Method Development and Validation of Sitagliptin Phosphate Monohydrate in Pure and Tablet Dosage Form by UV-Vis Spectroscopy."** By using methanol as a solvent and the proposed method is based on the principle that Sitagliptin exhibiting an absorption spectra of wavelength maxima 267 nm.
34. Gebremriam Ketema, *et al,* [67] **(2012)** reported **"Development and validation of RP-HPLC Method for Simultaneous Estimation of Sitagliptin and Simvastatin in Bulk and Tablet Dosage Forms."** A mobile phase consisting of a mixture of buffer: acetonitrile: methanol (40:35:25v/v), pH adjusted to 3.5 with orthophosphoric acid and Triethylamine. The mobile phase was filtered through a 0.45µ nylon filter, sonicated for 15 min and delivered at a flow rate of 1.0 ml/min. Analysis was performed at ambient temperature with detection at 254 nm.

35. A.B. Loni *et.al.*[68] **(2012)** reported **"Method development and validation for simultaneous determination of Sitagliptin phosphate and Metformin hydrochloride by RP-HPLC in bulk and tablet dosage form."** The separation of two drugs was achieved on Hi–Q Sil C–18 (250 mm × 4.6 mm) 5 µm columns, at the flow rate of 1.2 ml/min. The mobile phase consists of Acetonitrile: Methanol: phosphate buffer (pH 4) in the ratio of 20:30:50 v/v/v.
36. Shyamala.M *et al.*[69] **(2011)** reported **"Validated RP-HPLC for Simultaneous Estimation of Sitagliptin Phosphate and Metformin Hydrochloride in Tablet Dosage Form."** by using mobile phase consists of acetonitrile and phosphate buffer in the ratio of 45:55. The detection was carried out at a wavelength 260 nm
37. Parag Pathade *et al.* [70] **(2010)** reported **"Development and Validation of Stability indicating UV Spectrophotometric Method for the estimation of Sitagliptin Phosphate in bulk and tablet dosage form."** By using distilled water as a solvent and 267 nm was selected as maximum wavelength for absorption.
38. Radhika Bhaskar *et.al.*[71]**(2010)** reported **"Simultaneous Estimation of Simvastatin and Metformin Hydrochloride in Bulk and Solid Dosage Forms."** by using methanol as a solvent and the estimation of Simvastatin was carried out at 247 nm while Metformin hydrochloride was estimated at 232.2 nm.
39. P Bonde, S Sharma, *et al***.**[72] **(2010)** reported **"Development and Validated UV Spectrophotometric and RP-HPLC Methods for the Estimation of Simvastatin and Ezetimibe in Combined Pharmaceutical Dosage Form."** The optimized mobile phase comprising of acetonitrile: water (0.2% triethylamine) (70:30) (v/v) and detection was carried out at 247 nm. The percentage estimations of the Simvastatin and Ezetimibe in market formulations by UV

spectrophotometric was found in between 96.2-99.6% and by RP-HPLC was found in between 97.8-99.8%

Reported Methods for Simvastatin:

40. B.Stephen Rathinaraj *et al.* [73] **(2010)** reported **"Development and Validation of an HPTLC Method for the Estimation of Simvastatin and Ezetimibe."** The mobile phase used was a mixture of chloroform: benzene: methanol: acetic acid (6.0:3.0:1.0:0.1 v/v/v/v). The detection of spots was carried out at 250 nm.
41. Joshi H. V *et al.* [74] **(2010)** reported **"Simultaneous Derivative and Multicomponent Spectrophotometric Determination of Simvastatin and Ezetimibe in Tablets.** The methods employed are first order derivative spectrophotometry using zero crossing techniques and multicomponent analysis both the drugs obey the Beer's law in the concentration range employed for these methods.
42. A Sunitha *et al.* [75] **(2010)** reported **"Development and Validation of Spectrophotometeric Method for Simultaneous Determination of Simvastatin and Ezetimibe in Tablet Formulations."** By using methanol as a solvent and the absorbance values at 236 nm and 234 nm of over line spectrum was used for the estimation of Simvastatin and Ezetimibe respectively without mutual interference.
43. Mujeeb Ur Rahman *et al.* [76] **(2010)** reported **"Simultaneous estimation of Simvastatin and Ezetimibe in pharmaceutical tablet dosage forms by RP-HPLC."** Chromatographic separation was achieved isocratic ally phenomenax C18 column (250 x 4.6 mm i.d.) with a mobile phase composed of 75:20:5 of acetonitrile: methanol: orthophosphoric acid (0.1%) v/v/v at flow rate of 1 ml/min. Detection is carried out using a UV-vis detector at 238 nm. The retention time of

Simvastatin and Ezetimibe was found to be 3.701 min and 5.975 min. respectively.

44. Jayapal Reddy Samaa.C, Rama *et al.* [77] **(2010)** reported **"Simultaneous estimation of Simvastatin and Ezetimibe in pharmaceutical formulations by RP-HPLC method."** Chromatographic separation was achieved on a X-terra RP-18 column (50 × 4.6 mm, 5µ) using a mobile phase consisting of 0.05M phosphate buffer pH3.0 and Acetonitrile in the ratio of 45:55 at a flow rate of 0.8ml per minute. The detection was made at 236 nm. The retention time of Simvastatin and Ezetimibe were 3.3 and 0.8 minutes respectively.
45. Nagaraju P. *et al.* [78] **(2009)** reported **"A Validated Reverse Phase HPLC Method for the Simultaneous Estimation of Simvastatin and Ezetimibe in Pharmaceutical Dosage Forms."** By using Mobile Phase Consisted of Acetonitrile: Buffer (0.1% v/v Ortho Phosphoric acid, pH – 3) in the ratio of 75:25 v/v delivered at a flow rate of 1.5 ml / min and wavelength of detection at 238 nm.
46. Nilesh Jain *et al.* [79] **(2009)** reported **"Spectrophotometric method for simultaneous estimation of Simvastatin and Ezetimibe in bulk drug and its combined dosage form."** by using a combination of methanol and phosphate buffer (7.4 pH) in 7:3 ratios was selected as solvent. The estimation of Simvastatin was carried out by dual wavelength method at 223 nm and 254.5 nm while Ezetimibe was estimated as single component at 258.5 nm.
47. V. L. N. Seshagiri Rao *et al.* [80] **(2010)** reported **"Simultaneous Determination of Simvastatin and Ezetimibe in Tablets by HPLC."** using a mobile phase consisting of 0.01 M ammonium acetate buffer and acetonitrile (35:65 *v/v*) at a flow rate of 1 mL/min. The detection was made at 240 nm. The retention times for Ezetimibe and Simvastatin were 5.9 and 8.5 min respectively.

48. Nilesh Jain *et.al.* [81] **(2008)** reported **"RP- HPLC method for simultaneous estimation of Simvastatin and Ezetimibe in bulk drug and its combined dosage form."** by using 70% methanol as a solvent and mobile phase composed of methanol: water: acetonitrile in the ratio of 75: 18.75: 6.25 % v/v/v at flow rate of 1.8 ml/min. Detection is carried out using a UV - pda detector at 231 nm.
49. BG Chaudhari *et al.* [82] **(2010)** reported **"Determination of Simvastatin, Pravastatin sodium and Rosuvastatin calcium in tablet dosage forms by HPTLC."** The stationary phase used was precoated silica gel 60F 254.The mobile phase used was a mixture of chloroform: methanol: toluene (6:2:2, v/v/v). The method has been completely validated and proved to be rugged.

Reported Methods for Thiocolchicoside:

50. Vilas. D. Patil *et al.*[83] **(2012)** reported **"Spectrophotometric method for estimation of Thiocolchicoside and Diclofenac potassium in capsule dosage form by simultaneous equation method."** by using 0.1N NaOH as a solvent proposed method involves formation of 'simultaneous equations' at 259 nm and 277 nm.
51. Bhavin P Morelia *et al.* [84] **(2012)** reported "**Application of RP-HPLC Method for Simultaneous Estimation of Thiocolchicoside and Diclofenac in commercially available capsules."** by using mobile phase Acetonitrile: water (70:30 % v/v, adjusted at pH 3.0) at a flow rate of 1.0 ml/min and detection was performed at 258 nm.
52. Pravin O. Patil *et al.*[85] **(2012)** reported "**Validated RP- HPLC Method for Simultaneous Estimation of Thiocolchicoside & Etodolac in Bulk Drug and In Pharmaceutical Dosage Form."** The method was carried out on a Qualisil BDS

RP C-18 (250 mm x 4.6 mm, 5 μm) column with a mobile phase consisting of methanol: ammonium acetate buffer (85:15 *v/v*) pH adjusted to 3.2 with ortho-phosphoric acid of acetate buffer and flow rate of 1.0 mL min^{-1}. Detection was carried out at 223 nm. The retention time for THI and ETO was found to be 3.007 and 6.100 min, respectively.

53. Sunil R. Dhaneshwar *et al.* [86] **(2011)** reported **"Validated HPTLC Method for Simultaneous Estimation of Thiocolchicoside and Aceclofenac in Bulk Drug and Formulation." by** using the solvent system consisted of toluene: ethyl acetate: methanol: glacial acetic acid (4: 6: 2: 0.5 v/v/v/v). Densitometry evaluation of the separated zones was performed at 255 nm.
54. Arvind R Umarkar *et al.* [87] **(2011)** reported "**Stability Indicating RP- HPLC Method for Estimation of Thiocolchicoside in Capsule Dosage Forms."** by using a mobile phase consisting of acetonitrile: water (70:30) was used. The flow rate was 1.0 mL min-^{1} with UV detection at 286 nm.
55. Arvind R Umarkar *et al.* [88]**(2011)** reported **"Simultaneous Estimation of Thiocolchicoside and Diclofenac Potassium by UV Spectrophotometer Using Multi component Method"** and the detection of the constituents was done using UV detector at 254,259,265,271,286 for THC and DICP.
56. Jyoti Shrivastav *et al.* [89]**(2011)** reported **"Application of HPTLC in the Simultaneous estimation of Thiocolchicoside and Diclofenac in Bulk drug and pharmaceutical dosage form."** Chromatographic separation was performed on silica gel 60 F254 as the stationary phase and the toluene: acetone: methanol: formic acid (5:2:2:0.01 v/v/v/v) as mobile phase. Densitometric evaluation of the separated zones was performed at 280 nm. The two drugs were satisfactorily

resolved with Rf values of 0.29 ± 0.02 and 0.71 ± 0.02 for THIO and DICLO, respectively.

57. Sohan S. Chitlange *et al.* [90]**(2010)** reported **"Simultaneous estimation of Thiocolchicoside and Aceclofenac in pharmaceutical dosage form by spectrophotometric and LC method."** The first developed method is Area under curve method, wavelength range selected are 264.5-254.5 nm for Thiocolchicoside and 279.0-269.0 nm for Aceclofenac respectively. Second developed method is RP- HPLC method using Thermo C_{18} column (4.6 mm i.d. × 250 mm) and Acetonitrile: Water: 0.025M pot. Dihydrogen orthophosphate buffer (pH adjusted to 3.0 with orthophosphoric acid) in the ratio of 70:10:20 % v/v/v as mobile phase.
58. Krishna R Gupta *et al.* [91]**(2010)** reported **"Simultaneous UV-Spectrophotometric determination of Thiocolchicoside and Diclofenac in Pharmaceutical formulation."** Method which includes Simultaneous Equation method (Method I), Absorbance Correction method (Method II). For development of Method I, wavelengths were selected 260.0 nm λ max for Thiocholchicoside and 276.5 nm λ max for Diclofenac Sodium, while for Method II, 276.5 nm λ max for Diclofenac sodium and 373.0 nm is isoabsorptive point of Thiocholchicoside and Diclofenac sodium.
59. Shekhar M. Bhavsar, *et al.* [92] **(2010)** reported **"Validated RP-HPLC method for simultaneous estimation of Lornoxicam and Thiocolchicoside in solid dosage form."** The sample was analyzed using Buffer (5.7606 gm Ammonium Dihydrogen Phosphate in 2000 mL of milli- Q water, adjust pH 7.3 with Tri Ethyl Amine): Methanol in the ratio of 45:55, as a mobile phase at a flow rate of 1.5 mL min^{-1} and detection at 290 nm. The retention time for Lornoxicam and Thiocolchicoside was found to be 9.40 and 2.96 min respectively.

Reported Methods for Ketoprofen

60. **B. Tsvetkova,** *et al.* [93]**(2013)** reported **"HPLC Determination of Ketoprofen in Tablet Dosage Forms."** The chromatographic separation was achieved on a LiChrosorb C_{18}, 250 mm x 4.6 mm, 5 μm columns at a detector wavelength of 230 nm and a flow rate of 1.0 ml/min. The mobile phase was composed of methanol, 0.1M ammonium acetate buffer pH 6.9, acetonitrile and tetrahydrofuran (73:20:5:2 *v/v/v/v*). The retention time of ketoprofen was 3.49 min.
61. R. Deveswaran, *et al.* [94] **(2012)** reported **"Development of an Analytical Method for Spectrophotometric Estimation of Ketoprofen using Mixed Co Solvency Approach."** The present study demonstrates the use of mixed co solvency in the enhancement of solubility and estimation of ketoprofen, practically water insoluble dug and thus precludes the use of organic solvents. The selected solubilizers were sodium citrate (15%), PEG 400 (8%) and polyvinyl pyrolidine (7%). Beer's law was obeyed in the concentration range of 2-20 μg/ml at wavelength of 256 nm.
62. Veena Nair**,** *et al.* [95] **(2010)** reported **"A simple spectrophotometric estimation of Ketoprofen in tablets using mixed hydrotropy."** A novel, safe and sensitive method of spectrophotometric estimation in the ultraviolet region has been developed using a mixed hydrotropic solution, containing a blend of 30% w/v urea, 13.6% w/v sodium acetate and 11.8% w/v sodium citrate for the quantitative determination of ketoprofen, a poorly water soluble drug, in tablet dosage form.
63. A. Mohammad., *et al.*[96] **(2010)** reported **"Identification of Ketoprofen in Drug Formulation and Spiked Urine Samples by Micellar Thin Layer Chromatography and its Quantitative Estimation by High Performance Liquid Chromatography."** The proposed method involves use of amino acid

impregnated silica gel layers as stationary phase with mixed micelles (0.5% aqueous solutions of sodium dodecyl sulphate plus Triton X-100 and acetone (8:5:1.5, v/v) as mobile phase. The HPLC determination of ketoprofen (formulated and spiked urine) samples carried out at l=270 nm with mobile phase comprising of acetonitrile: double distilled water: acetic acid (1:1:1, v/v).

64. Zholt Kormosh, *et al.* [97] **(2009)** reported **"Spectrophotometric Determination of Ketoprofen and Its application In Pharmaceutical Analysis."** The method is based on the reaction of ketoprofen with an analytical reagent n Astra Phloxin FF n at pH 8.0 n 10.8 and followed by the extraction of formed ion associate in toluene with spectrophotometric detection (it has an absorption maximum at 563 nm, ε = 7.6 × 104 L×mol-1×cm-1). The calibration plot was linear from 0.8 n 16.0 μg×mL-1 of ketoprofen.

Reported Methods for Desloratadine:

65. Rima M. Bankar *et al.* [98]**(2013)** reported **"Simultaneous Estimation of Montelukast Sodium and Desloratadine by Ratio Spectra Derivative Spectrophotometry Method in Combined dosage forms."** by using methanol as the solvent. Both the drugs showed linearity in the range of 5-40 μg/ml.
66. R.B.Patel *et.al.* [99] **(2012)** reported **"Validation of Stability Indicating High Performance Liquid Chromatographic Method for Estimation of Desloratadine in Tablet Formulation." by** using a mixture of methanol–phosphate buffer of pH 7.0 (70:30 v/v) as a mobile phase with an UV detector at 254 nm. Desloratadine stock solution was subjected to different stress conditions. The degraded product peaks were well resolved from the pure drug peak with significant difference in their retention time values.

67. Rele rajan. V *et al.*[100]**(2012)** reported **"A Simple Extractive Spectrophotometric determination of Loratadine, Desloratadine and Rupatadine From Pharmaceutical Formulations."** and the method was based on the formation of colored ion pair complexes by the drugs with thiocynate ions and The absorbance values were measured at 618 nm, 614 nm and 616 nm respectively.
68. Ektha Sharma *et al.*[101] **(2012)** reported "**Development and Validation of First Order Derivative Spectrophotometric Method for Simultaneous Estimation of Ambroxol Hydrochloride and Desloratadine Hydrochloride in Combined Tablet Dosage Form."** by using 0.1N Hydrochloric acid as a solvent and the determinations were made at 256 nm (ZCP of Desloratadine Hydrochloride) for Ambroxol Hydrochloride and 308 nm (ZCP of Ambroxol Hydrochloride) for Desloratadine Hydrochloride.
69. E.A. Sharma *et al.*[102] **(2012)** reported **"Development and Validation of Dual Wavelength Uv Spectrophotometric Method For Simultaneous Estimation of Ambroxol Hydrochloride and Desloratadine Hydrochloride in their Combined Tablet Dosage Form."** by using 0.1N Hydrochloric acid as a solvent and the principle for dual wavelength method is "the absorbance difference between two points on the mixture spectra is directly proportional to the concentration of the component of interest. The method was based on determination of Ambroxol Hydrochloride at the absorbance difference between 253.2 nm and 258.5 nm and Desloratadine Hydrochloride at the absorbance difference between 301.2 nm and 314 nm.
70. Ektha Sharma *et al.* [103] **(2012)** reported **"Development and Validation of First Order Derivative Spectrophotometric Method for simultaneous estimation of Ambroxol hydrochloride and Desloratadine hydrochloride in combined tablet**

dosage form" by using 0.1N Hydrochloric acid as a solvent and the determinations were made at 256 nm (ZCP of Desloratadine Hydrochloride) for Ambroxol Hydrochloride and 308.4 nm (ZCP of Ambroxol Hydrochloride) for Desloratadine Hydrochloride.

71. Sharma Ekta A. e*t al.*[104] **(2012)** reported **"Development and Validation of High Performance Thin Layer Chromatography Method for Simultaneous Estimation of Ambroxol Hydrochloride and Desloratadine Hydrochloride in Combined Tablet Dosage Form."** By using the solvent system of Chloroform: Ethyl Acetate: Methanol: Triethyl Amine (6: 4.5: 2.5: 0.8, v/v/v/v). Densitometric evaluation of separated zones was performed at 245 nm.

72. Vibhuti R. Chhatrala *et al.* [105]**(2012)** reported **"Simultaneous Estimation of Montelukast Sodium and Desloratadine by RP-HPLC in their Marketed Formulation."** By using reversed-phase C-18 column (250 mm × 4.8 mm i.d., particle size 5 μm) column with mobile phase consisting of methanol: water: Acetic acid (90:10:0.05 v/v/v) and effluents were monitored at 280 nm.
73. SV Patel *et al.* [106]**(2012)** reported **"Development and Validation of Derivative Spectroscopic Method for Simultaneous Estimation of Montelukast Sodium and Desloratadine in Bulk and combined Dosage Form."** by using methanol as a solvent. The quantification was achieved by the first-order derivative spectroscopy method at 297.20 nm and 339.20 nm over the concentration range of 3-38 μg/ml for estimation of Desloratadine (r^2 = 0.9993) and 6-36 μg/ml Montelukast (r^2=0.9999) in a combined tablet formulation.
74. Navneet Kumar *et al.*[107] **(2011)** reported **"A Validated Stability-Indicating RP-UPLC Method For Simultaneous Determination of Desloratadine and**

Sodium Benzoate in Oral Liquid Pharmaceutical Formulations." The chromatographic separation was achieved on Acquity BEH C8 (100 mm x 2.1 mm) 1.7 μm column by using mobile phase containing a gradient mixture of solvent A (0.05 M KH2PO4 and 0.07 M triethylamine, pH 3.0) and B (50:25:25 v/v/v mixture of acetonitrile, methanol and water) at flow rate of 0.4 mL/min. Column temperature was maintained at 40°C and detection was carried out at a wavelength of 272 nm.

75. Satish Bondili *et al.* [108] **(2011)** reported **"Spectroscopic Method for Determination of Desloratidine in Bulk and Its Tablet Dosage Form."** by using methanol as a solvent and its absorbance is measured at 242 nm.

Reported Methods for Ambroxol Hydrochloride:

76. Umadevi. B *et al.* [109] **(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.
77. Nagavalli. D *et al.* [110] **(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.
78. Jain P.S.1 *et al.* [111] **(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.

79. 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.
80. Deshpande *et al.* [112] **(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 densitometric 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 254 nm.
81. Senthil Raja. M *et al.*[113] (**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 2 ml/min using PDA detection at 220 nm.

82. Makarand Avhad *et al*. [114]**(2009)** reported **"Development and validation of Simultaneous UV-spectrophotometric method for the determination of Levofloxacin and Ambroxol in tablets."** The method involves Q-absorbance equation at 219 nm isoabsorptive point and at 287 nm using distilled water as a solvent

83. Krishna Veni Nagappan *et al.* [115] **(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.

84. Neela M Bhatia *et al.* [116] **(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).

85. Lakshmana prabhu. S *et al.* [117] **(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 242 nm and 231 nm.

86. Pai PNS *et al.* [118] **(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.

87. Meiling Qi *et al.* [119] **(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 220 nm.
88. Dincer *et al.* [120] **(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 255 nm. 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).
89. Kuchekar. B.S *et al.* [121] (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 500 nm and by using Ferric nitrate and Nitric acid produced yellowish orange chromogen at 400 nm.
90. Francisco G *et al.* [122] **(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**.**

91. Narayana reddy. M *et al.* [123] **(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 Doxofylline:

92. Giriraj P *et al.* [124] (**2011)** reported **"Simultaneous Estimation and Method Validation of Montelukast Sodium and Doxofylline in Solid Dosage form by RP-HPLC."** The developed Reverse-Phase High Performance Liquid Chromatographic method was carried out on inertsil C_{18} column with mobile phase comprising of Acetonitrile: Methanol: Ammonium acetate buffer, pH 5.5 (10:70:20) at a flow rate of 1.5 ml/min. The Spectrophotometric detection was carried out at 274 & 347nm.
93. Akhilesh G *et al.* [125] **(2011),** reported **"Method Development and Acid Degradation Study of Doxofylline by RP-HPLC and LC-MS/MS."** The developed and validated Reverse Phase High Performance Liquid Chromatography used acetonitrile: 0.05M formic acid in the ratio of 90:10, pH 3.0 as mobile phase and monitored at 274 nm. The acid degradation product as well as pathway was characterized by LC-MS/MS.
94. Venkatesan S, *et al.* [126] **(2011),** reported **"A Simple HPLC Method for Quantitation of Doxofylline in Tablet Dosage Form.''** The quantitation was carried out using inertsil octyl decyl column. The mobile phase was Methanol: Water [30:70v/v]. The LOD and LOQ are found to be 5.152µg/ml and 15.97µg/ml respectively. The flow rate was 1.5 ml/min with UV detection at 274 nm.

95. Joshi HR *et al.* [127] **(2010),** reported **"Spectrophotometric and Reversed Phase High-Performance Liquid Chromatographic Method for the Determination of Doxofylline in Pharmaceutical Formulations."** The methods employed are 1. Ultraviolet Spectrophotometric Determination and 2. High Performance Liquid Chromatography. In UV-Spectrophotometric method, the absorbance was measured at 274 nm. The developed Reverse Phase High Performance Liquid Chromatographic method used Hypersil ODS C_{18} column (250 X 4.6 mm, 5 mm) and the mobile phase consisting of potassium dihydrogen phosphate (pH 3.0 ± 0.2): acetonitrile in the ratio of 80:20, at a flow rate of 1.0 ml/min, and detected at 210 nm.

96. Revathi R *et al.* [128] **(2011),** reported **"High Performance Liquid Chromatographic Method Development for Simultaneous Analysis of Doxofylline and Montelukast Sodium in a Combined Form."** The chromatographic analysis was performed on inertsil C_8 column (4.6 mm X 250 mm, 5 μm) in isocratic mode with mobile phase consisting of Methanol-Sodium phosphate buffer (75:25), pH 6.5 at a flow rate of 1 ml/min. The eluents were detected at 230 nm.

97. Maulik Oza *et al.* [130] **(2012),** reported **"Development and Validation of Solvent Extraction Spectrophotometric Method for Simultaneous Estimation of Doxofylline and Terbutaline sulphate in their Combined Dosage Form."** UV 2080 plus model, silicon photodiode detector controlled by UV Analyst software was utilized in this method. Solvent extraction method was performed at 277 nm and 279 nm for Doxofylline in chloroform and Terbutaline sulphate in water respectively.

98. Gadapa Nirupa *et al.* [131] **(2012),** reported **"Novel LC Method Development and Validation for Simultaneous Determination of Montelukast and Doxofylline in Bulk and Pharmaceutical dosage form."** The chromatographic separation was carried out on $_{C18}$ column (150 mm X 4.6 mm, 5 μm) with the mobile phase comprised of methanol-phosphate buffer, pH 4.5 (90:10) at a flow rate of 1 ml/min and the eluents were detected at 280 nm

99. Atkuru Veera *et al.* [132] **(2011),** reported **"Development and Validation of Novel Analytical Methods for Estimation of Doxofylline in Bulk and Dosage Forms."** Three methods were developed. The first method is based on charge-transfer complex formation of the drug with p-chloranilic acid and second method involves the formation of colored chloroform extractable ion-pair complex of the drug with bromophenol blue under acidic condition. The third method is based on ternary complex formation of the drug with molybdenum (V) thiocyanate binary complex. The colored products are quantitated spectrophotometrically at 540 nm, 390 nm and 690 nm for first, second and third method respectively. Development and validation of novel analytical methods for estimation of Doxofylline in bulk and dosage forms

100. Lakshmi Sivasubramanian *et al.* [133] **(2011),** reported **"RP-HPLC and HPTLC Methods for Determination of Doxofylline in Bulk and Formulations."**The developed HPLC method used acetonitrile and methanol (70:30) as mobile phase on Intersil C_{18} Column (4.6 X 250 mm), at a flow rate of 1 ml/min and monitored at 208 nm. In HPTLC method, silica gel 60 Merck pre-coated plates was used, with mobile phase comprised of acetonitrile and methanol (7:3), and detected at 208 nm.

101. Amit Kumar De *et al.* [134] **(2012)** reported, **"Development and Validation of Same RP-HPLC Method for Separate Estimation of Theophylline and Doxofylline in Tablet Dosage Forms."** The method was carried out in isocratic mode using X terra column (4.6 150 mm. i.d., 5µm, C18) with a mobile phase composed of phosphate buffer (5.3 mM, pH 3.5)and acetonitrile in the ratio of 60:40 (v/v) at a flow rate of 0.5 ml/min. The chromatographic analysis with ultraviolet detection was monitored at 271nm for Theophylline and at 274 nm for Doxofylline.

102. Ashu Mittal *et al.* [135] **(2010),** reported **"Development and Validation of Rapid HPLC Method for Determination of Doxofylline in Bulk Drug and Pharmaceutical Dosage Forms."** The chromatographic separation was achieved on HiQ Sil C_{18} column using a mobile phase of acetonitrile: buffer (50: 50), pH 3, at a flow rate of 1 ml/min with detection of analyte at 272 nm. The separation was achieved within 3.1 ± 0.3 min for Doxofylline.

103. Narendra G. Patre *et al.* [136] **(2009),** reported **"A Validated, Stability-Indicating HPTLC Method for Analysis of Doxofylline".** The developed method used aluminum plates coated with silica gel 60 F_{254} as stationary phase and toluene-methanol (8:2) as mobile phase, followed by densitometric measurement at 254 nm. The R_F value of Doxofylline was 4.3. The drug was subjected to acidic, alkaline, oxidative, and photolytic stress to establish a validated stability-indicating HPTLC method

Reported Methods for Salbutamol Sulphate:

104. Mukesh Maithani *et al.*[137] **(2012),** reported **"Development and Validation of a Stability-Indicating HPLC Method for the Simultaneous Determination of**

Salbutamol Sulphate and Theophylline Pharmaceutical Dosage Forms." A reversed-phase phenomenax C-18 column (250 mm × 8 mm i.d., particle size 10 μm) column with mobile phase consisting of acetonitrile and phosphate buffer 65:35 (v/v) (pH 4.2 ± 0.02, adjusted with triethylamine) was used. The flow rate was 1.2 mL min-1 and effluents were monitored at 235 nm. The retention times (Rt) of Salbutamol sulphate and Theophylline were found to be 5.33 min and 13.36 min, respectively.

105. Selvadurai Muralidharan *et al.* [138] **(2012),** reported **"High Performance Liquid Chromatographic Method Development and Its Validation for Salbutamol."** Chromatographic separation achieved isocratic ally on reversed-phase c18 Colum (250 × 4.6 mm, 5μ) and the column effluent was monitored by UV detector at 276 nm. The mobile phase used was acetonitrile: 50mm ammonium acetate (ph 7.0), (80: 20 % v/v) at a flow rate of 1.0 ml/min.

106. Sagar Suman Panda *et al.* [139] **(2010**), reported **"Difference UV spectrophotometric method for estimation of levosalbutamol sulphate in tablet dosage form."** This spectrophotometric method is based on the principle that levosalbutamol sulphate shows two different forms in acidic and basic medium that differ in the absorption spectra in basic and acidic medium.

107. Arun K. Mishra *et al.* [140] **(2012),** reported **"Validated UV spectroscopic method for estimation of Salbutamol from tablet formulations."** The wavelength maxima (λ max) for Salbutamol were found to be 276 nm. The linearity for this method was found to be in the range of 10- 120 μg/ml.

108. **Deepak Kumar Jain** *et al.* [141] **(2012),** reported **"Simultaneous determination of Salbutamol sulphate and Doxophylline in tablets by reverse phase liquid chromatography."**Chromatographic separation achieved isocratic ally on Luna

C1column (5 µm, 150mm x 4.60mm) and Acetonitrile/KH2PO4 buffer (40:60, v/v, pH 3.0 with OPA) as mobile phase, at a flow rate of 0.5 ml/min. Detection was carried out at 225 nm.

109. N Jyothi *et al.* [142] **(2012)** reported **"Development and Validation of an HPLC method for the Simultaneous Estimation of the Salbutamol Sulphate and Ipratropium in Inhalation Dosage Forms."** Good sensitivity for all analytes was observed with UV detection at wavelength of 226 nm, Separation was performed on a Symmetry C18 (4.6 X 150mm) 5µm,using a mixture of 0.05M phosphate buffer pH 3.5 and methanol in the ratio of (400:600, v/v).

110. Pangal Anees *et al.* [143]**(2013),** reported **"Simple Titrimetric Method for the Estimation of Salbutamol Sulphate (SBS in Pharmaceutical Formulations."** In titrimetry, aqueous solution of Salbutamol sulphate is treated with a measured excess of NBS in acetic acid medium and after the oxidation of SBS is complete, the unreacted oxidant is determined iodometrically. In this method the amount of NBS reacting corresponds to the amount of SBS content

AIM

&

PLAN OF WORK

3.1 AIM OF WORK

Most of Pharmaceutical companies are manufacturing multiple drug formulations to meet the market demand and patient compatibility. It is a well known fact that a combination of drugs has wider range to treat ailments as compared to the single drug component. Very few analytical methods are available for estimation of multiple drug formulation by simultaneous methods. This simultaneous estimation was less time consuming and usage of solvent is minimized, UV, HPLC grade of solvents used for respective determinations and the solvent should be readily available and cheaper. The solvent should be completely extracting the active ingredient from formulation. Several methods were reported for the estimation of those combinations individually as well as in combination with some other drugs.

The combined dosage form selected for the present study containing those following combinations in tablets, recently these combinations of the drugs introduced in to the market.

1. TOLPERISONE HYDROCHLORIDE AND PARACETAMOL

2. SIMVASTATIN AND SITAGLIPTIN PHOSPHATE

3. THIOCOLCHICOSIDE AND KETOPROFEN

4. DESLORATADINE AND AMBROXOL HYDROCHLORIDE

5. DOXOFYLLINE AND SALBUTAMOL SULPHATE

The non - availability of UV spectrophotometry, HPLC, HPTLC methods until now for simultaneous analysis of combination made it a worthwhile objective to pursue the present

work. Hence the present work, aim to develop a simple, precise and accurate methods for estimation of those combination in combined dosage form and to validate the developed method by UV Spectrophotometry, RP-HPLC, and HPTLC.

3.2 PLAN OF WORK

SURVEY ON LITERATURE

The survey on literature performed for above combinations for their physiochemical properties, solubility and pharmacology and for analytical techniques. So this basic information gives notion for method development.

In the present work, simple, accurate, precise, repeatable, rugged and reproduce, method developed for the estimation of above mentioned combination in tablet dosage form by UV, RP-HPLC and HPTLC.

For UV Method.

- Find the Drugs solubility in various solvents
- To determine maximum absorbance and overlaid the spectra
- Determining the standard absorbance for all selected wavelength for each drugs.
- Development of simple, precise, accurate and sensitive method, in the specified range
- Validation of developed methods as per ICH guidelines

For RP-HPLC method.

- Selection of suitable mobile phase for suitable for two drugs based on resolution and capacity factor.
- Selection of wavelength
- Selection of p^H
- Development of chromatogram in formulation

- Validation of the development method

For HPTLC Method

- Determination of suitable detection wavelength
- Optimization of chromatographic conditions
- Analysis of formulation
- System suitability testing

Validation

The method to be developed should be validated as per ICH guidelines. The various parameters of validation are Linearity, Range, Precision, Accuracy, LOD, LOQ and Ruggedness

MATERIALS &METHODS

4. MATERIALS AND METHODS

4.1 TOLPERISONE HYDROCHLORIDE AND PARACETAMOL

Drug samples (Raw material)

Pharmaceutically pure sample of Tolperisone Hydrochloride and Paracetamol was obtained as a souvenir samples from Amaranth Pharma Ltd. Pondicherry, India.

Formulation used

Tablet MYODCALM-A containing 500 mg of Paracetamol, 150 mg of Tolperisone Hydrochloride. The tablet was purchased from a local Pharmacy.

Chemicals and solvents used

Methanol (AR grade), Methanol (HPLC grade), Water for HPLC, Acetonitrile (HPLC grade) were purchased from Qualigens India Pvt**.** Ltd. and Loba Chemie India Ltd.

Instruments used

Different instruments used to carry out the present work, are

1. Shimadzu AUX - 220 Digital balances.
2. Sonicator – Sonica ultrasonic cleaner – model 2200 MH.
3. Centrifuge apparatus.

   PERKIN ELMER FT - IR
4. SHIMADZU – 1700 Double Beam - UV – Visible spectrophotometer with pair of 10 mm matched quartz cells
5. SHIMADZU HPLC

LC – 10 ATVP solvent deliver module

SPD – 10 A_{VP} UV – VIS detector

ELICO SL – 210 double beam, UV - Visible spectrophotometer with pair of 10mm matched quartz cells

6. ELICO pH meter (Model LI - 120)
7. Melting point apparatus - Guna enterprises Chennai
8. Micropipette

4.1.2 Specifications (Terms) of instruments[25]

Shimadzu AUX- 220 digital balance

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

b) Double Beam UV- Visible spectrophotometer [26]

Model: Shimadzu, UV- 1700; Double beam UV-Visible spectrophotometer.

ELICO SL – 210; Double beam UV-Visible spectrophotometer.

Specification	**Shimadzu UV-1700**	**Elico SL -210**
Light source	20 W halogen lamp, Deuterium lamp, Light source position automatic adjustment mechanism. Built in lamp lighting time display function.	Tungsten halogen lamp (W), Deuterium lamp (D), Light source position automatic adjustment mechanism.
Monochromator	Aberration- correcting concave blazed holographic grating	Concave holographic grating with 1200 lines/ mm
Detector	Silicon photodiode	Photodiode
Stray light	0.04% or less (220 nm; NAI 10g/lt) 0.04% or less (340 nm; $NaNo_2$ 50g/lt).	<0.05% T at 220 nm with NAI 10g/ lt
Measurement wavelength range	190 ~ 1100 nm	190 ~1100 nm
Spectral band width	1 nm or less (190 to 900 nm).	1.8 nm
Wave length accuracy	± 0.5 nm on broad automatic wavelength calibration mechanism.	± 0.5 nm automatic wavelength calibration mechanism.
Recording range	Absorbance; - 3.99 ~3.99 Abs	Absorbance; ± 3.000 Abs

	Transmittance; - 399 ~ 399%	
Photometric accuracy	± 0.004 Abs (at 1.0 Abs). ± 0.002 Abs (at 0.5 Abs).	± 0.005 Abs (at 1.0 Abs). ± 0.010 Abs (at 0.5 Abs).
Operating temperature/ Humidity	Temperature range; 15 to 35˚C Humidity range; 35 to 80% (15 to below 35˚C) 35 to 70% (30 to below 35˚C)	Temperature range; 15 to 35˚C Humidity range; 35 to 80% (15 to below 35˚C) 35 to 70% (30 to below 35˚C)

C) **Shimadzu HPLC**

Detector specifications	
Light source	Deuterium lamp
Wavelength range	190 to 600 nm
Spectral band width	8 nm
Wavelength accuracy	± 1 nm
Wavelength reproducibility	± 0.1 nm
Cell path length	10 mm
Cell volume	8 µl
Operating temperature range	4 to 35°C (39 to 104°F)
Recording range	Can be set between 0.0001 and 2.56 AUFS in 0.0001 AUFS steps

Pump specifications	
Pump type	Double reciprocating plunger pump
Pumping methods	Constant flow delivery and constant pressure delivery
Suction filter	10 μm mesh
Line filter	5 μm mesh, capacity 30 μl approximately
Operating temperature	4 to 35°C

4.2. METHODS EMPLOYED

An attempt was made to develop and validate simple, precise and accurate methods for the estimation of Tolperisone Hydrochloride and Paracetamol in pure form and in combined tablet dosage form by,

1. UV Spectrophotometric method

- Simultaneous equation method
- Absorbance Ratio method
- Area under Curve method
- Derivative method

2. RP – HPLC.

3. HPTLC.

4.2.1.1 UV SPECTROPHOTOMETRIC METHODS

Selection of solvent

The solubility of drugs was determined in a variety of non - polar to polar solvents as per I.P. specification**.** The common solvent was found to be distilled water for the analysis of Tolperisone Hydrochloride and Paracetamol for the proposed method.

Preparation of standard stock solution

Accurately weighed drug samples of both Tolperisone Hydrochloride (20 mg) and Paracetamol (30 mg) were transferred into a suitable standard volumetric flask separately, dissolved and diluted up to a mark with distilled water. Both the drug solutions were diluted so as to get 10 µg/ml. The solution were scanned in the UV region of 200-400 nm in 1cm cell against distilled water as blank and the overlaid spectra was recorded.

Selection of analytical wavelength for estimation

From the overlaid spectra, by the observation of spectral characteristics of TPE and PCL, were estimated simultaneously by simultaneous equation method. The wavelengths selected were λ max of both drugs i.e., 261 nm and 243 nm. For the Absorption ratio method, the absorbance are measured at two wavelengths one being the λ max of one of the component Paracetamol, λ_2 243 nm and the other being a equal absorptive of two component λ_1 Tolperisone Hydrochloride, 254 nm an isobestic point. For the Area under curve method, the wavelength selected were 253 nm – 269 nm for TPE and 274 nm – 284 nm for PCL. For Derivative spectroscopic methods, the zero order spectrum was derivatised to first order, $\Delta\lambda$ = 1 nm for the entire spectrum and the wavelength 261 nm was selected for the estimation of PCL, which is the zero crossing point for TPE and 243 nm was selected for the estimation of TPE which is zero crossing point for PCL**.**

Preparation of calibration graph

Appropriate volumes of aliquots from standard stock solutions were transferred into different volumes of 10 ml capacity. The volume was adjusted to the mark and suitably diluted so as to get the final concentration range 0.5- 2.5 ml of 10 μg/ml of TPE and 0.5-2.5 ml of 60 μg/ml of PCL. Absorbances of these solutions were recorded in the respective wavelengths.

Synthetic Mixture

From the standard stock solution, 0.5 ml - 2.5 ml of 10 μg/ml and 0.5 ml - 2.5 ml of 60 μg/ml solution were transferred into 10 ml volumetric flask to get a concentration of 0.5-2.5 μg/ml and 3-15 μg/ml respectively. The absorbance of the prepared synthetic mixtures was measured at the selected wavelengths. The amount of drugs in the prepared synthetic mixture was calculated.

Quantification of formulation

The Tablet MYODCALM - A containing 500 mg of Paracetamol, 150 mg of Tolperisone Hydrochloride was obtained for all analytical study. Twenty tablets of formulation were weighed accurately. The average weight of tablets were found and powdered. The tablet powder equivalent to 30 mg of PCL was weighed and transferred into a 100 ml volumetric flask, added a minimum quantity of distilled water to dissolved the substance by using ultra sonication for 15 minutes and made up to the volume with the same. The content was filtered through Whatmann filter paper No. 41. Filtrate was suitably diluted to get a final concentration, to obtain 9 μg mL^{-1} of PCL which contains 1.5 μg mL^{-1} of TPE theoretically. The absorbance of sample solution was measured at all

selected wavelengths. The content of TPE and PCL in sample solution of tablet was calculated. This procedure was repeated for six times.

Recovery studies

In order to ensure the reliability and suitability of the proposed method, recovery studies were carried out. It was done by mixing known quantity of standard drug with formulation sample and the content were pre analyzed by the proposed method. To a quantity of formulation equivalent to 30 mg of PCL and standard drugs PCL and TPE were added at 80%, 100% and 120% levels. The drugs were extracted, diluted and re analyzed as per the formulation procedure. Absorbance was noted at the respective wavelength. The amount of each drug recovered from the formulation was calculated for all the drugs by Simultaneous Equation method, Absorbance ratio method, Area under curve method and Derivative spectroscopic method. This accuracy estimation was repeated in triplet in each concentration.

Validation of developed method

Validation of analytical method is the process to establish by laboratory studies that the performance characteristic of the method meets the requirements for the intended analytical application. Performance characteristic are expressed in terms of analytical parameters.

Linearity

The linearity of the method is its ability to elicit test results that are directly proportional to the concentration of the analyte in samples. To establish the linearity of the method, six separate series of solutions were prepared in the concentration range of 0.5 to

2.5 μg mL^{-1} of TPE, and PCL in the concentration range of 3 to 15 μg mL^{-1} at 243 nm, 261 nm, 254 nm 253 nm - 269 nm for TPE and 274 nm - 284 nm for PCL. A calibration curve was plotted as concentration vs. absorbance for the described above methods.

Precision

The repeatability of the method was confirmed by the formulation analysis, repeated in six times with the same concentration. The amount of each drug present in the tablet formulation was calculated. The percentage 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, respectively. The amount of drugs was determined and % RSD was also calculated.

Ruggedness

Ruggedness of the method was confirmed by the analysis of formulation performed in different instrument and also by the different analysts. The amount and % RSD were calculated.

Accuracy

Accuracy of the method was confirmed by recovery studies. To the pre analyzed formulation, known quantities of raw materials of TPE and PCL 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.

LOD and LOQ

The linearity study was carried out for six times. The LODs and LOQs of developed method were studied as per ICH Guidelines. Several approaches for determining the LODs and LOQs are possible, depending on the procedure i.e. a non-instrumental or instrumental.

$$LODs = 3.3\ \sigma/S$$

$$LOQs = 10\ \sigma/S$$

Where σ = standard deviation of response, s = slope of calibration curve

The LOD and LOQ were calculated by using the average of slope and standard deviation of response (Intercept).

4.2.1.2 REVERSE PHASE – HPLC METHOD

In Present investigation, developed a simple and sensitive RP-HPLC method for quantitative estimation of Tolperisone Hydrochloride and Paracetamol in bulk drug Pharmaceutical formulations.

Selection of chromatographic method

Proper selection of the method depends upon the nature of sample, polarity, molecular weight, Pka value and solubility. Tolperisone Hydrochloride and Paracetamol dissolved in polar solvent; hence RP-HPLC was selected to estimate them. So, Reverse Phase Chromatographic technique was selected by using C_{18} column as a stationary phase with different ratios of Acetonitrile and Methanol as a mobile phase.

Preparation of mobile phase

500 ml of Acetonitrile and 500 ml of Methanol was accurately measured, mixed and ultra sonicated for 15 minutes.

Method development and optimization of chromatographic conditions

Solutions of Tolperisone Hydrochloride and Paracetamol (10 μg mL^{-1}) were prepared in the mobile phase [Acetonitrile: Methanol (50:50 v/v)], scanned in the UV region of 200 - 400 nm and recorded the spectra. It was found that both drugs have marked absorbance at 254 nm and can be effectively used for estimation of two drugs without interference. Therefore, 254 nm was selected as detection wavelength for estimation of two drugs by RP - HPLC method with an isocratic elution technique.

Stability check

The absorbance of the solutions of Tolperisone Hydrochloride and Paracetamol (10 μg mL^{-1}) in mobile phase was checked for their stability at 254 nm and it was found that two drugs were stable up to 5 hour and 30 minutes.

Initial separation conditions

The following chromatographic conditions were preset initially to get better resolution of Tolperisone Hydrochloride and Paracetamol.

Mode of operation	-	Isocratic
Stationary phase	-	C_{18} column (150 mm × 4.6 mm i.d. 5μ)
Mobile phase	-	Acetonitrile: Methanol

Proportion of mobile phase - 50: 50 % v/v

Detection wavelength - 254 nm

Flow rate - 1 ml/ min

Temperature - Ambient

Sample load - 20 µl

Operating pressure - 121 kgf

Method - External standard calibration method.

The mobile phase was primarily allowed to run for 30 minutes to record a study baseline. TPE and PCL were injected individually and the respective chromatogram was recorded. It was found that TPE peak was broader. For this reason different ratios of mobile phase with different solvents were tried to obtain good chromatogram with acceptable system suitability parameters.

Selection of mobile phase

Different mixtures of mobile phase with different ratios were selected and their chromatograms were recorded, they include the following:

S.No	MOBILE PHASE	OBSERVATION
1.	Acetonitrile: Methanol: (50: 50 v/v)	TPE and PCL were eluted with tailing.
2.	Acetonitrile: Methanol: (40: 60 v/v)	TPE and PCL were eluted with fronting with broad peak.
3.	Acetonitrile: Methanol: (30: 70 v/v)	TPE and PCL were eluted with tailing but the resolution was poor.

4.	Acetonitrile: Methanol: (40: 60: 0.1 ml of 0. 1% Triethylamine v/v)	Both peaks eluted were broad with capacity factor less than 1.

From the above information, the mobile phase of Acetonitrile: Methanol (40: 60: 0.1 ml of 0.1% Triethylamine v/v) these two drugs were eluted with sharp peak and better resolution. Hence this mobile phase was used.

Effect of ratio of mobile phase

The different ratios of Acetonitrile: Methanol: 0.1ml of 0.1% Triethylamine (40: 60 v/v) ratio was selected; the peaks obtained were very sharp with better resolution. Hence this ratio was selected for the analysis of TPE and PCL

Conditioning of the column

Before the new run of HPLC, conditioning of the column was done by passing HPLC grade methanol at 1ml/min flow rate for 30 min, so as to remove the remains of the previous runs present in the column.

Optimized chromatographic conditions

The following optimized conditions were employed for analysis of TPE and PCL Isocratic RP – HPLC method.

Mode of operation - Isocratic

Stationary phase - C_{18} column (150 mm × 4.6 mm I'd. 5µ)

Mobile phase - Acetonitrile: Methanol: 0.1 ml of 0.1% Triethylamine

Proportion of mobile phase - (40: 60: v/v)

Detection wavelength - 254 nm

Flow rate - 1 ml/ min

Temperature - Ambient

Sample load - 20 µl

Operating pressure - 121kgf

Method - External Standard Calibration method.

Preparation of standard stock solution

25 mg of TPE and 25 mg of PCL was weighed accurately and transferred into a 25 ml volumetric flask, dissolved in methanol and the volume was made up to the mark with methanol (1000 µg mL^{-1}), and further dilution was done to acquire a final concentration of 40 µg mL^{-1} and 20 µg mL^{-1} solution respectively.

Linearity and calibration curve

The primary stock solutions (1 –5 ml of 20 µg mL^{-1}) were transferred into 10 ml volumetric flasks and made up to the mark with the mobile phase, containing the concentrations of 2, 4, 6, 8 and 10 µg mL^{-1} of TPE. The primary stock solution (1 –5 ml of 40 µg/ml) was transferred into 10 ml volumetric flasks and made up to the mark with mobile phase, containing the nominal concentrations of 4, 8, 12, 16 and 20 µg $mL^{-1}$of PCL. 20 microliters of this solution was injected each time into a column at a flow rate of 1ml/min. The detection of the method was monitored at 254 nm. The procedure was

repeated in triplet. The peak areas were plotted against concentration and the calibration curve was constructed.

Quantification of formulation

Twenty tablets of formulation (MYO-MR-PLUS) containing TPE 150 mg, PCL 325 mg were accurately weighed. The average weight of tablets was found and crushed to a fine powder. From the triturate of 20 tablets, a mass equivalent to 30 mg of Paracetamol was accurately weighed and transferred into a 50 ml volumetric flask and added a minimum quantity of methanol to dissolve the substance and the solution was sonicated for 30 minutes and made up to the volume with the same. The solution was filtered through Whatmann filter paper No. 41. From the filtrate, further dilutions were completed with mobile phase. The solution was expected to contain 12 μg mL^{-1} of PCL and 6 μg mL^{-1} of TPE .This solution was used for further analysis. A steady base line was recorded with optimized chromatographic conditions. After the stabilization of base line for 30 minutes, six test solutions of formulation were injected and recorded the chromatograms. The concentration of each test solution was determined by using slope and intercept values from the calibration graph.

Recovery studies

Preparation of raw material stock solutions of TPE and PCL

12 mg of TPE and 24 mg of PCL were weighed accurately and transferred into a 100 ml volumetric flask and dissolved in methanol, the volume was made up to the mark with methanol (240 μg mL^{-1}). Further dilution was made to acquire a concentration of 48 μg mL^{-1} of TPE and 96 μg mL^{-1} of PCL respectively.

Recovery Procedure

The recovery experiment was done by adding known concentrations of TPE and PCL raw material to the 50 % pre-analyzed formulation. Standard TPE and PCL in the range of 80 %, 100 % and 120% were added to the 50% pre-analyzed formulation**.** To each 1 ml of pre analyzed formulation solution (12 μg $mL^{-1}$and 6 μg mL^{-1}) added 1, 1.25, 1.5 ml of 48 μg mL^{-1} raw material stock solution of PCL and 1, 1.25, 1.5 ml of 96 μg mL^{-1} raw material of TPE into a 10 ml volumetric flasks and made up to the mark with mobile phase. The procedure was repeated as per analysis of formulation in triplet of each concentration. The quantity of drug recovered was calculated by using slope and intercept values from the calibration graph.

System suitability

A system suitability test of the chromatographic system was performed before each validation run. Five replicate injections of standard preparation were injected. Asymmetry, theoretical plate, resolution and % RSD of peak area were determined. Acceptance criteria for system suitability, Asymmetry not more than 2.0, theoretical plate not less than 1800 and % RSD of peak area not more then 2.0, were full filled during all validation parameter.

VALIDATION OF THE DEVELOPED METHODS

Validation of the developed method was carried out as per ICH Guidelines. Parameters such as Linearity, Accuracy, Precision, Specificity, Robustness, LODs and LOQs were taken up as tests for method validation.

Linearity

For Quantitative analysis of TPE and PCL linearity curve was plotted. Linearity range of TPE and PCL was established in the concentration range of 2 -10 μg $mL^{-1}$of TPE 4 -20 μg mL^{-1} respectively. The slope and intercept along with its correlation coefficient was calculated.

All the validation procedure is similar as spectroscopic method.

4.2.1.3 HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY METHOD

In HPTLC, the separation of the components of a mixture is based on the principle of adsorption. The HPTLC differ from the TLC in the size of silica gel used as the stationary phase and automated sampling application and detection. In the present study a twin trough chamber and silica 60 F 254 were used. The standard and sample solutions of Tolperisone Hydrochloride and Paracetamol were spotted and the chromatograms were observed in Iodine chamber. The following mixture of solvents were tried to optimize the mobile phase chamber.

Choice of Mobile Phase

TRAIL NO	MOBILE PHASE	RATIO
1.	Chloroform : Ammonia : Methanol	6 : 1 : 3
2.	Chloroform : Methanol : Acetic acid	6 : 3 : 1
3.	Acetonitrile : Ethyl acetate	9 : 1
4.	Methanol : Acetic acid :Water	5 : 3 : 2
5.	Chloroform : Methanol :Water	6 : 3 : 1

6.	Chloroform : Acetic acid :Water	6:2:2
7.	Toluene: Ethyl acetate :water	7:2:1

The mobile phase chosen after trail was Chloroform: Acetic acid: Water (6:2:2) due to its better resolution.

Optimization of Variants in TLC

The composition of mobile phase, chamber saturation (equilibration time), plate equilibration time, the distance of solvent development and band width of the spot are a few variants which affect the Rf values of drugs.

Optimized Chromatographic Conditions

Stationary phase : Silica gel 60-F_{254} aluminum sheets

Mobile phase : Chloroform: Acetic acid: Water

Mobile phase ratio : 6:2:2 % V/v/v

Detection Wavelength : UV detection at 264 nm

Development mode : Ascending mode

Temperature : 60° C

Development chamber : Twin trough chamber

Preparation of standard stock solution

Accurately weighed sample of both TPE and PCL each of 25 mg was weighed accurately and transferred into a 25 ml volumetric flask, dissolved in methanol and the

volume was made up to the mark with methanol (1000 μg mL^{-1}). Further dilution was made to acquire a final concentration of 40 μg mL^{-1} and 20 μg mL^{-1}solution respectively.

Linearity and calibration curve

The primary stock solutions (1-5 ml of 20 μgmL^{-1}) were transferred into 10 ml volumetric flasks and made up to the mark with mobile phase containing the nominal concentration of 20 -100 ngμL^{-1} of TPE and 40 - 200 ngμL^{-1} of PCL. The procedure was repeated in triplet. The peak areas were plotted against concentration and the calibration curve was constructed.

Quantification of formulation

As similar to HPLC method, the solutions were prepared and further dilutions were made to obtain 120 ngμL^{-1} solution which contains 60 ngμL^{-1} of TPE solution theoretically. This solution was used for further analysis. 1 μl spots were placed on the plates and the chromatogram was developed in the twin trough chamber. From the peak area the amount of drug present were calculated. The procedure was repeated for six times. The concentration of each test solution was determined by using slope and intercept values from the calibration graph

Recovery studies

As similar to HPLC method, the solutions were prepared. To each 1 ml of pre analyzed formulation solution (12 μg $mL^{-1}$and 6 μg mL^{-1}). Added 1, 1.25,1.5 ml of 48 ng μL^{-1} raw material stock solution of Paracetamol and 1, 1.25, 1.5 ml of 96 ng μL^{-1} of TPE into a 10 ml volumetric flasks and made up to the mark with mobile phase. The procedure was repeated as per analysis of formulation in triplet of each concentration. The quantity of

drug recovered was calculated by using slope and intercept values from the calibration graph.

VALIDATION OF THE DEVELOPED METHODS

Validation of the developed method was carried out as per ICH Guidelines. Parameters such as Linearity, Accuracy, Precision, Specificity, Robustness, LODs and LOQs were taken up as tests for method validation.

Linearity

For Quantitative analysis of TPE and PCL linearity curve was plotted. Linearity range of TPE and PCL was established in the concentration range of (20-100 ng/µl, 40-200 ng/µl,) respectively. The slope and intercept along with its correlation coefficient was calculated.

All the validation procedure is similar as spectroscopic method.

4.2.2 SIMVASTATIN AND SITAGLIPTIN PHOSPHATE

Drug samples (Raw material)

Pharmaceutically pure sample of Simvastatin and Sitagliptin Phosphate were generously gifted as a souvenir samples from Alkem laboratories Ltd. Hyderabad, India.

Formulation used

Juvisync tablets containing 40 mg of Simvastatin, 100 mg of Sitagliptin phosphate was procured from a local Pharmacy.

An attempt was made to develop and validate versatile, precise and accurate methods for the estimation of Simvastatin and Sitagliptin phosphate pure form and in combined tablet dosage form by,

1. UV Spectrophotometric method

    - Derivative spectrophotometric method

2. RP – HPLC.

3. HPTLC.

4.2.2.1 UV SPECTROPHOTOMETRIC METHOD

Selection of solvent

The solubility of drugs was determined in a variety of non polar to polar solvents as per I.P. specification**.** The common solvents were found to be as methanol for the analysis of Simvastatin and Sitagliptin phosphate for the proposed method.

Preparation of primary stock solution

Primary stock solutions were prepared by dissolving 20 mg of Simvastatin and 25 mg Sitagliptin phosphate separately and diluted using methanol as a solvent to get a concentration of 1000 $\mu gmL^{-1}$and further dilution was completed to get concentration of 10 $\mu g\ mL^{-1.}$

Selection of analytical wavelength

The selection of wavelength for the estimation of SIM and SITA was done by preparing a suitable dilute stock solution containing 10 µg mL^{-1} concentration solutions. The stock solutions were scanned between the wavelength ranges from 200 - 400 nm by using methanol as blank and the spectrum was recorded.

Derivative spectrophotometric method

From the overlaid spectra, by the observation of spectral characteristics of SIM and SITA simultaneous equation method was not possible, since Sitagliptin Phosphate showed less absorbance. The zero order spectra obtained and it was derivatised to first order spectrum. The values of amplitudes were measured for Sitagliptin Phosphate at 277 nm (zero crossing point for Simvastatin) and 238 nm measured for Simvastatin (zero crossing point for Sitagliptin Phosphate) respectively**.** A calibration curve was constructed and regression equation was obtained for each drug. The Stability was performed by measuring the absorbance of same solution at different time intervals. It was observed that SITA and SIM were stable for 6 hours.

Linearity Characteristics

An appropriate aliquots of stock solution of SITA (0.5 - 2.5ml of 200 µg/ml) and SIM (1-5ml of 40 µg/ml) were transferred into 10 ml volumetric flasks to get the concentration of 10-50 µg/ml, 4-20 µg/ml and made up to the volume with methanol. The zero order spectra were derivatized to first order derivative spectra with the wavelengths 238 nm, 277 nm (zero crossing points for SITA and SIM) respectively**.** A calibration curve was constructed and regression equation was obtained for each drug.

Synthetic Mixture

From the standard stock solution, 0.5 ml - 2.5 ml of 200 μg/ml and 1ml - 5 ml of 40 μg/ml solution were transferred into 10 ml volumetric flask to get a concentration of 10-50 μg/ml and 4-20 μg/ml respectively. The absorbance of the prepared synthetic mixtures was measured at the selected wavelengths. The amount of drugs in the prepared synthetic mixture was calculated.

Analysis of sample formulation

Twenty tablets (JUVISYNC) were weighed accurately and made into a fine powder. A mass equivalent to 30 mg of SITA was weighed and transferred into a 100 ml volumetric flask, added a minimum quantity of methanol to dissolved the substance by using ultra sonication for 15 minutes, and made up the volume to 100 ml volumetric flask. Then the content was filtered through Whatmann filter paper No. 41. The solution was expected to contain 30 μg mL^{-1} of SITA and 12 μg mL^{-1} of SIM. The absorbance measurements were made 6 times for the formulation by derivatising the zero order spectra into first order derivative spectra at 238 nm, 277 nm respectively.

Recovery studies

In order to ensure the reliability and suitability of the proposed method, recovery studies were carried out. It was done by mixing known quantity of standard drug with formulation sample and the content were pre analyzed by the proposed method. To a quantity of formulation equivalent to 30 mg of SITA and standard drugs SITA and SIM were added at 80%, 100% and 120% levels. The drugs were extracted diluted and re

analyzed as per the formulation procedure. Absorbance was noted at respective wavelength. Recovery studies were repeated for three times and the results were shown.

VALIDATION OF THE DEVELOPED METHODS

Validation of the developed method was carried out as per ICH Guidelines. Parameters such as Linearity, Accuracy, Precision, Specificity, Robustness, LODs and LOQs were taken up as tests for method validation.

Linearity

For Quantitative analysis of Sitagliptin Phosphate and Simvastatin, linearity curve was plotted. Linearity range of Sitagliptin Phosphate and Simvastatin were established in the concentration range of (10-50 µg/ml, 4-20 µg/ml) respectively. All validated procedures are followed as per first method.

4.2.2.2 REVERSE PHASE – HPLC METHOD

In RP – HPLC, Proper selection of the method depends upon the nature of sample, polarity, molecular weight, Pka value and solubility. The drugs Sitagliptin phosphate and Simvastatin, for the present study were polar. So, Reverse Phase Chromatographic technique was selected by using C_{18} column as a stationary phase with different ratio of Acetonitrile and Methanol as a mobile phase.

Preparation of mobile phase

400 ml of Acetonitrile and 500 ml of Methanol was accurately measured, mixed and ultra sonicated for 15 minutes to degas the mobile phase.

Method development and optimization of chromatographic conditions

Solutions of Sitagliptin Phosphate and Simvastatin (10 µg/ ml) were prepared in the mobile phase [Acetonitrile: Methanol (40:50 v/v)] were scanned in the UV region of 200 - 400 nm and recorded the spectra. It was found that both drugs have marked absorbance at 251 nm and can be effectively used for estimation of two drugs without interference. Therefore, 251 nm was selected as detection wavelength for estimation of two drugs by RP - HPLC method with an isocratic elution technique.

Stability check

The absorbance of the solutions of Sitagliptin Phosphate and Simvastatin, (10 µg/ml) in mobile phase was checked for their stability at 251 nm and it was found that two drugs are stable for around six hour and 30 minutes.

Optimization of chromatographic conditions

Initial separation conditions

The following chromatographic conditions were preset initially to get better resolution of Sitagliptin phosphate and Simvastatin.

Mode of operation - Isocratic

Stationary phase - C_{18} column (150 mm × 4.6 mm i.d. 5µ)

Mobile phase - Acetonitrile: Methanol

Proportion of mobile phase - 40: 50 v/v

Detection wavelength - 251 nm

Flow rate - 1 ml/ min

Temperature - Ambient

Sample load - 20 µl

Operating pressure - 121 kgf

Method - External standard calibration method.

The mobile phase was primarily allowed to run for 30 minutes to record a study baseline Sitagliptin Phosphate and Simvastatin were injected individually and the respective chromatogram was recorded. It was found that Simvastatin peak was broader and tailing. For this reason different ratios of mobile phase with different solvents were tried to obtain good chromatogram with acceptable system suitability parameters.

Selection of mobile phase

Different mixtures of mobile phase with different ratios were selected and their chromatograms were recorded, they include the following.

S.No	Mobile phase	Observation
1.	Acetonitrile: Methanol :water (40:50:10v/v/v)	Sitagliptin Phosphate and Simvastatin were eluted with tailing.
2.	Acetonitrile: Methanol: water (30:60:10) v/v/v)	Sitagliptin Phosphate and Simvastatin were eluted with fronting with broad peak.
3.	Acetonitrile: Methanol: (40: 60 v/v)	Sitagliptin Phosphate and Simvastatin were eluted with tailing but the resolution was poor.
4.	Acetonitrile: Methanol:	Both peaks eluted were broad with capacity

	(50:50:0.1ml of 0.1% Triethylamine v/v)	factor less than 1.
5.	Acetonitrile: Methanol: (70:30:0.1ml of 0.1% Triethylamine v/v)	Both peaks eluted were broad with capacity factor tailing.
6.	Acetonitrile: Methanol: (40:60:0.1ml of 0.1% Triethylamine v/v)	Both peaks eluted were sharp with capacity factor less than 1.

From the above information, in the mobile phase of Acetonitrile: Methanol (40: 60: 0.1ml of 0.1 % Triethylamine v/v) these two drugs were eluted with sharp peak and better resolution. Hence this mobile phase was used to optimize the chromatographic conditions.

Effect of ratio of mobile phase

The different ratios of Acetonitrile: Methanol (40: 60 V/v): 0.1ml of 0.1 % Triethylamine was selected; the peaks obtained were very sharp with better resolution. Hence this ratio was selected for the analysis of Acetonitrile: Methanol: 0.1 ml of 0.1 % Triethylamine (40: 60 v/v)

Optimized chromatographic conditions

The following optimized conditions were employed for analysis of Sitagliptin Phosphate and Simvastatin by Isocratic RP – HPLC method.

Mode of operation - Isocratic

Stationary phase - C_{18} column (150 mm × 4.6 mm i.d. 5µ)

Mobile phase - Acetonitrile: Methanol: 0.1ml of 0.1 % Triethylamine

Proportion of mobile phase - (40: 60: v/v)

Detection wavelength - 251 nm

Flow rate - 1 ml/ min

Temperature - Ambient

Sample load - 20 µl

Operating pressure - 121 kgf

Method - External Standard Calibration method.

Preparation of standard stock solution

50 mg of Sitagliptin phosphate and 20 mg of Simvastatin was weighed separately and transferred into a 50 ml volumetric flask and dissolved in methanol, after dissolution the volume was made up to the mark with methanol (1000 µg/ml and 400 µg/ml) respectively.

Linearity and calibration curve

The aliquots of standard stock solution (4 –6 ml of 1000 µg/ml and 400 µg/ml) were transferred into 25 ml volumetric flasks and made up to the mark with mobile phase, containing the concentrations of 160-240 µg/ml Sitagliptin phosphate and Simvastatin 64-96 µg/ml respectively. All the solutions of 20 µl were injected and the chromatograms were recorded at 251 nm. The procedure was repeated in triplet. The peak areas were plotted against concentration and the calibration curve was constructed.

Quantification of formulation

Estimation of Sitagliptin Phosphate and Simvastatin in tablet formulation by RP - HPLC was carried out using optimized chromatographic conditions. Twenty tablets of formulation (JUVISYNC) were accurately weighed; the average weight of tablets was found and crushed to a fine powder. From the triturate of 20 tablets, an amount equivalent to 100 mg of Sitagliptin Phosphate was accurately weighed and transferred into a 50 ml volumetric flask and added a minimum quantity of methanol to dissolve the substance and the solution was sonicated for 30 minutes and made up to the volume with the same (2000 µg/ml) and filtered through Whatmann filter paper No.41. From the clear solution, further dilutions were made by diluting 2.5 ml into 25 ml volumetric flask, and further with mobile phase to obtain 200 µg/ ml of SITA and 80 µg/ml of SIM theoretically. This solution is used for further analysis. A steady base line was recorded with optimized chromatographic conditions. After the stabilization of base line for 30 minutes, six test solutions of formulation were injected and recorded the chromatograms. The concentration of each test solution was determined by using slope and intercept values from the calibration graph.

Recovery Procedure

The recovery experiment was done by adding known concentrations of SITA and SIM raw material to the 50 % pre-analyzed formulation. Standard SIM and SITA in the range of 80 %, 100 % and 120% are added to the 50% pre-analyzed formulation**.** To each 2.5 ml of pre analyzed formulation solution (200 µg/ml and 80 µg/ml) added 4, 5, and 6 ml of 1000 µg /ml and 400 µg/ml raw material stock solution of Sitagliptin phosphate into 25 ml volumetric flasks and made up to the mark with mobile phase. The procedure was repeated as per analysis of formulation in triplet of each concentration. The quantity of

drug recovered was calculated by using slope and intercept values from the calibration graph.

System suitability studies

The system suitability studies were conceded as per ICH guidelines. The parameters like capacity factor, tailing factor, asymmetry factor and number of theoretical plate and resolution were calculated.

4.2.2.3 HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY METHOD

In HPTLC, the separation of the components of a mixture is based on the principle of adsorption. The HPTLC differ from the TLC in the size of silica gel used as the stationary phase and automated sampling application and detection. In the present study a twin trough chamber and silica 60 F 254 were used.

Selection of Stationary Phase

The resolution of SITA and SIM was achieved using TLC plate made up of silica gel G60 F 254 coated on an Aluminium support (E.Merck).The size of the silica gel particle was 2 μm and thickness of sorbent layer was 0.2 mm. The plates were supplied in 20 × 10 cm size which was cut in to appropriate sizes for method development.

Selection of Mobile Phase

The mobile phase system was chosen based on the solubility and polarity of two drugs. The solution of drugs was prepared in methanol and used for spotting. Methanol gets vaporized soon after application on to the plate under nitrogen stream. After trying different mobile phase system an ideal system was chosen based on the resolution between

compounds. The fixed mobile phase system for the separation of two drugs with an appropriate R_f values. The drugs were scanned at 255 nm after the development.

The velocity of mobile phase in HPTLC is affected by the nature of the stationary phase (porosity, packing, particle size, etc), as well as mobile phase properties (viscosity, surface tension, vapour pressure of solvents, etc). Generally the velocity of mobile phase decreases during chromatographic development due to higher resistance of stationary phase densely packed with fine particles.

Various mobile phase tried were

Mobile Phase	**Ratio**
Methanol: Acetonitrile: Glacial acetic acid	(10: 6: 0.01% v/v/v/)
Ethyl Acetate: Chloroform: Methanol: 25% ammonia	(6: 3:1 % v/v/v/)
Ethyl acetate: Methanol: 25% ammonia: Glacial Acetic acid	(7.5: 1.5:1% v/v/v/)
Toluene: Benzene: Methanol	(5: 3: 2 % v/v/v)
Benzene: Toluene: Diethyl amine	(5: 3: 2 % v/v/v)
Toluene : Methanol: Ammonia	(5: 4:1 % v/v)
Toluene: Methanol : Acetic acid	(5: 4: 1 % v/v)
Toluene: Methanol: Acetic acid	(4: 3: 3% v/v)

From the above list of mobile phase Toluene: methanol: acetic acid (5: 4: 1 % v/v/v) was found to be an ideal mobile phase with good resolution between the spots with the R_f value 0.5241 for SITA and 0.7865 for SIM respectively.

Optimization of Variants in TLC

The composition of mobile phase, chamber saturation (equilibration time), plate equilibration time, the distance of solvent development and bandwidth of the spot are the few variants which affect Rf values of drugs.

Chamber Saturation (Equilibration time)

Chamber saturation is done so that equilibration is established eventually between the components of developing solvents and their vapour phase and the formation of secondary solvent fronts could be avoided.

Hence in the current study chamber saturation was taken in to consideration to achieve reproducible Rf values and peak area. The mobile phase was placed on one side of twin trough chamber and shaken well. Different saturation times were maintained for different mobile phase. The chamber saturation time for Toluene: methanol: acetic acid (5: 4: 1 % v/v/v) was 30 minutes.

Selection of Detection Wavelength

By comparing the spectral characters of SITA and SIM, 255 nm the detection wavelength selected for the method with reference to the spectral confirmation graph.

Optimized Chromatographic Conditions

After conforming with the mobile phase and detection wavelength, the optimized conditions for the method was as follows

Stationary Phase	-	Silica Gel 60 F 254 HPTLC Plates
Mobile Phase	-	Toluene: methanol: acetic acid
Mobile Phase ratio	-	(5: 4: 1 % v/v/v)
Detection	-	CAMAG TLC scanner 3, at 255 nm

Temperature	-	Room Temperature
Chamber	-	Twin trough Chamber
Development Mode	-	Ascending Mode

Preparation of Standard Stock Solution

20 mg of Sitagliptin phosphate was weighed accurately and transferred into a 100 ml volumetric flask and dissolved in methanol, after dissolution the volume was made up to the mark with methanol (200 ng/µl). 25 mg of Simvastatin were weighed accurately and transferred into a 100 ml volumetric flask and dissolved in methanol, after dissolving, the volume was made up to the mark with methanol (1000 µg/ml). Further dilution was made by pipetting 4 ml of mother solution into same 100 ml standard flask to acquire a concentration of 40 ng/µl solution.

Linearity and Calibration Curve

The aliquots of standard stock solution (0.5 ml – 2.5 ml of 200 ng/µl) were transferred into 10 ml volumetric flasks and made up to the mark with mobile phase, containing the concentrations of 100 -500 ng/µl Sitagliptin phosphate. The standard stock solution of Simvastatin (1 – 5 ml of 400 ng/µl) was transferred into 10 ml volumetric flasks and made up to the mark with mobile phase, contains 40-200 ng/µl SIM respectively. All the solutions were injected and the chromatograms were recorded at 255 nm.

Quantification of Formulation

As similar to Derivative method, the solutions were prepared and further dilutions were made by diluting 1 ml into 10 ml and further dilution was made with mobile phase to obtain 300 ng/µl of SITA which contain 120 ng/µl of SIM theoretically. This solution is

used for further analysis. A steady base line was recorded with optimized chromatographic conditions. After the stabilization of base line for 30 minutes, six test solutions of formulation were injected and recorded the chromatograms. The concentration of each test solution was determined by using slope and intercept values from the calibration graph.

Recovery studies

To each 1 ml of pre analyzed formulation solution (300 ng/µl and 120 ng/µl) added 1, 1.25, 1.5 ml of 24 ng/µl raw material stock solution of Sitagliptin phosphate and 1,1.25,1.5 ml of 96 ng/µl raw material of Simvastatin into a 10 ml volumetric flasks and made up to the mark with mobile phase. The procedure was repeated as per analysis of formulation in triplet of each concentration. The quantity of drug recovered was calculated by using slope and intercept values from the calibration graph.

Validation of Developed Method

Linearity

A calibration curve was plotted with concentration versus the peak area. The linearity range was checked in the concentration range of 100 –500 ng/ µl and 40 – 200 ng/ µl, of SITA and SIM respectively. The drugs were found to be linear in the specified concentration ranges.

As similar to Derivative method, a same validation procedure was followed

4.2.3 THIOCOLCHICOSIDE AND KETOPROFEN

Drug samples (Raw material)

Pharmaceutically pure sample of Thiocolchicoside and Ketoprofen were generously gifted as a souvenir samples from Alkem laboratories Ltd. Hyderabad, India.

Formulation used

RELAXEN - 4 containing 50 mg of Ketoprofen and 4 mg of Thiocolchicoside. The tablet was procured from a local Pharmacy.

An attempt was made to develop and validate versatile, precise and accurate methods for the estimation of Thiocolchicoside and Ketoprofen pure form and in combined tablet dosage form by,

1. UV Spectrophotometric method

    - Derivative spectrophotometric method

2. RP – HPLC

4.2.3.1 UV SPECTROPHOTOMETRIC METHODS

Selection of solvent

The solubility of drugs was determined in a variety of non polar to polar solvents as per I.P. specification**.** The common solvents were found to be as methanol and water for the analysis of the proposed method.

Preparation of standard stock solution

Standard stock solutions were prepared by dissolving 100 mg of Ketoprofen and 20 mg of Thiocolchicoside, diluted using methanol and water as solvent to get a concentration of 1000 μg mL^{-1} and further dilution was made to get concentration of 10 μg $mL^{-1.}$

Selection of analytical wavelength

The selection of wavelength for the estimation of THI and KET was done by preparing a suitable diluted stock solution containing 10 µg mL^{-1} concentration solutions. The stock solutions were scanned between the wavelength ranges from 200 - 400 nm by using water as blank and the spectrum was recorded.

Derivative spectrophotometric method

From the overlaid spectra, the zero order spectra obtained and it was derivatised to first order spectrum. The values of amplitudes were measured for THI at 233.5 nm (zero crossing point of KET) and 259 nm measured for KET (zero crossing point of THI) respectively**.**

Linearity Characteristics

An aliquots of stock solution of KET (1-5 ml of 500 µg/ml) and THI (1-5 ml of 40 µg/ml) were transferred into 10 ml volumetric flasks to get the nominal concentration in the range of 50 –300 µg/ml, 4 - 20 µg/ml were made up to the volume with water. The zero order spectra were derivatized to first order derivative spectra with the wavelengths 235.5 nm, 259 nm (zero crossing points for THI and KET) respectively**.** A calibration curve was constructed and regression equation was obtained for each drug.

Synthetic Mixture

From the standard stock solution, KET (1-5 ml of 500 µg/ml) and THI (1-5 ml of 40 µg/ml) solution were transferred to 10 ml volumetric flask to get a concentration of 50–300 µg/ml, 4-20 µg/ml and respectively. The absorbances of the prepared synthetic mixtures

were measured at the selected wavelengths. The amount of drugs in the prepared synthetic mixture was calculated.

Analysis of sample formulation

Twenty tablets (RELAXEN - 4) were weighed accurately and made into a fine powder. A quantity of tablet powder equivalent to 100 mg of Ketoprofen was weighed and transferred into a 50 ml volumetric flask, added a minimum quantity of methanol to dissolved the substance by using ultra sonication for 15 minutes, and completed the volume to 50 ml into a volumetric flask. Then the content was filtered through Whatmann filter paper No.41. From the cleared solution, the solution was expected to contain 100 μgmL^{-1} of KET and 8 μgmL^{-1} of THI. The absorbance measurements were made 6 times for the formulation by derivatising the zero order spectra into first order derivative spectra at 259 nm, 235.5 nm respectively.

Recovery studies

In order to ensure the reliability and suitability of the proposed method, recovery studies were carried out. It was done by mixing known quantity of standard drug with formulation sample and the content were Pre analyzed by the proposed method. To a formulation equivalent to 100 mg of KET and standard drugs, KET and THI were added at 80%, 100% and 120% levels. KET & THI was extracted, diluted and re analyzed as per the formulation procedure. Absorbances were noted at respective wavelength. Recovery studies were repeated for three times and the results are shown.

VALIDATION OF THE DEVELOPED METHODS

Validation of the developed method was carried out as per ICH Guidelines. Parameters such as Linearity, Accuracy, Precision, Specificity, Robustness, LODs and LOQs were taken up as tests for method validation.

Linearity

For Quantitative analysis of Thiocolchicoside and Ketoprofen, linearity curve was plotted. Linearity range of Thiocolchicoside and Ketoprofen was established in the concentration range of (50 - 300 µg/ml, 4 - 24 µg/ml) respectively.

All validated parameters are followed as per first method

4.2.3.2 REVERSE PHASE – HPLC METHOD

HPLC uses high pressure to force solvent through closed column containing very fine particles that give high resolution separations. The technique is used to separate and to determine species in variety of organic, inorganic and biological materials.

The drugs Thiocolchicoside and Ketoprofen for the present study were polar. So, Reverse Phase Chromatographic technique was selected by using C_{18} column as a stationary phase with different ratio of Acetonitrile and water as a mobile phase.

Preparation of mobile phase

700 ml of Acetonitrile and 300 ml of Water was accurately measured, mixed and ultra sonicated for 15 minutes to degas the mobile phase.

Method development and optimization of chromatographic conditions

Solutions of Thiocolchicoside and Ketoprofen (10 µg/ml) were prepared in the mobile phase Acetonitrile: Water (70: 30 v/v) and scanned in the UV region of 200 - 400 nm and recorded the spectra. It was found that both drugs have marked absorbance at 300 nm and can be effectively used for estimation of two drugs without interference. Therefore, 300 nm was selected as detection wavelength for estimation of two drugs by RP - HPLC method with an isocratic elution technique.

Stability check

The absorbance of the solutions of Thiocolchicoside and Ketoprofen (10 µg/ml) in mobile phase was checked for their stability at 300 nm and it was found that two drugs are stable for around five hours.

Optimization of chromatographic conditions

Initial separation conditions

The following chromatographic conditions were preset initially to get better resolution of Thiocolchicoside and Ketoprofen.

Mode of operation - Isocratic

Stationary phase - C_{18} column (150 mm × 4.6 mm i.d. 5µ)

Mobile phase - Acetonitrile: Water

Proportion of mobile phase - 70: 30 v/v

Detection wavelength - 300 nm

Flow rate - 1 ml/ min

Temperature - Ambient

Sample load - 20 µl

Operating pressure - 121 kgf

Method - External standard calibration method.

The mobile phase was primarily allowed to run for 30 minutes to record a study baseline Thiocolchicoside and Ketoprofen were injected individually and the respective chromatogram was recorded. It was found that Thiocolchicoside and Ketoprofen peak was broader and tailing. For this reason different ratios of mobile phase with different solvents were tried to obtain good chromatogram with acceptable system suitability parameters.

Selection of mobile phase

Different mixtures of mobile phase with different ratios were selected and their chromatograms were recorded, they include the following.

S.No	Mobile phase	Observation
1.	Acetonitrile : Water (70:30 v/v)	Thiocolchicoside and Ketoprofen were eluted with tailing.
2.	Acetonitrile : Methanol (60:40 v/v)	Thiocolchicoside and Ketoprofen were not eluted
3.	Acetonitrile : Water (50: 50 v/v)	Thiocolchicoside and Ketoprofen were eluted with tailing but the resolution was poor.
4.	Acetonitrile : Water: (30:70v/v)	Both peaks eluted were broad with capacity factor less than 1.
5.	Acetonitrile : Water (60:40v/v/)	Both peaks eluted were sharp with capacity

		factor less than 1.
6.	Acetonitrile : Methanol: water (30:50:20/v v/v)	Both peaks eluted were sharp with tailing.

From the above information, the mobile phase of Acetonitrile: Water (60: 40 v/v) these two drugs were eluted with sharp peak and better resolution. Hence this mobile phase ratio was used to optimize the chromatographic conditions.

Optimized chromatographic conditions

The following optimized conditions were employed for analysis of Thiocolchicoside and Ketoprofen by Isocratic RP – HPLC method.

Mode of operation - Isocratic

Stationary phase - C_{18} column (150 mm × 4.6 mm i.d. 5µ)

Mobile phase - Acetonitrile: Water

Proportion of mobile phase - (60: 40: v/v)

Detection wavelength - 300 nm

Flow rate - 1 ml/ min

Temperature - Ambient

Sample load - 20 µl

Operating pressure - 121 kgf

Method - External Standard Calibration method.

Preparation of standard stock solution

125 mg of Ketoprofen and 25 mg of Thiocolchicoside was weighed separately and transferred into a 50 ml volumetric flask and dissolved in methanol, after dissolution the volume was made up to the mark with methanol (2500 µg/ml and 500 µg/ml) respectively

Linearity and calibration curve

The aliquots of standard stock solution (4-6 ml of 2500 µg/ml and 1.6-2.4 ml of 500 µg/ml) were transferred into 25 ml volumetric flasks and made up to the mark with mobile phase. And further dilutions were made by taking 5 ml from the above stock and made up to 25 ml with mobile phase to get the concentration (80-120 µg/ml) of Ketoprofen and Thiocolchicoside (6.4–9.6 µg/ml) respectively. From all the solutions of 20 µl were injected and the chromatograms were recorded at 300 nm. The peak areas were plotted against concentration and the calibration curve was constructed

Quantification of formulation

Twenty tablets of formulation RELAXEN - 4 were accurately weighed; the average weight of tablets was found and crushed to a fine powder. From the triturate of 20 tablets, an amount equivalent to 250 mg of Ketoprofen was accurately weighed and transferred into a 50 ml volumetric flask and added a minimum quantity of methanol to dissolve the substance and the solution was sonicated for 30 minutes made up to the volume with the same (5000 µg/ml) and filtered through Whatmann filter paper No. 41. From the clear solution, further dilutions were made by diluting 5 into 25 ml volumetric flask, and further dilution was made with mobile phase to obtain 100 µg/ml of KET and 8 µg/ml of THI theoretically. This solution is used for further analysis. A steady base line was recorded with optimized chromatographic conditions. After the stabilization of base line for 30

minutes, six test solutions of formulation were injected and recorded the chromatograms. The concentration of each test solution was determined by using slope and intercept values from the calibration graph.

Recovery Procedure

The recovery experiment was done by adding known concentrations of THI and KET raw material to the 50% pre-analyzed formulation. Standard THI and KET in the range of 80 %, 100 % and 120% are added to the 50% pre-analyzed formulation**.** To each 5 ml of pre analyzed formulation solution (100 µg/ml and 8 µg/ml) added 5, ml of 400, 500 and 600 µg/ ml raw material stock solution of KET and 32, 40 and 48 µg/ml raw material stock solution of THI into 25 ml volumetric flasks and made up to the mark with mobile phase. The procedure was repeated as per analysis of formulation in triplet of each concentration. The quantity of drug recovered was calculated by using slope and intercept values from the calibration graph.

System suitability studies

The system suitability studies were conceded as per ICH guidelines. The parameters like capacity factor, tailing factor, asymmetry factor and number of theoretical plate and resolution were calculated.

VALIDATION OF THE DEVELOPED METHODS

Validation of the developed method was carried out as per ICH Guidelines. Parameters such as Linearity, Accuracy, Precision, Specificity, LODs and LOQs were taken up as tests for method validation.

Linearity

For Quantitative analysis of Ketoprofen and Thiocolchicoside, linearity curve was plotted. Linearity range of Ketoprofen and Thiocolchicoside was established in the concentration range of 80-120 μg/ml of Ketoprofen and (6.4–9.6 μg/ml) Thiocolchicoside respectively.

All validated procedures were followed as per first method.

4.2.4. DESLORATADINE AND AMBROXOL HYDROCHLORIDE

Drug samples (Raw material)

DESLORATADINE and AMBROXOL HYDROCHLORIDE bulk powder was kindly gifted by Micro labs Pharmaceuticals Ltd. Bangalore, India.

The commercial fixed dose combination product DYL - AX (AMB – 75 mg, DES – 5 mg) was procured from the local market which is manufactured by Ajanta Pharma Limited.

An attempt was made to develop, validate versatile, precise and accurate methods for the estimation of DESLORATADINE AND AMBROXOL HYDROCHLORIDE pure form and in combined tablet dosage form by,

UV Spectrophotometric method

- Absorption Ratio method
- Derivative spectrophotometric method

4.2.4.1 UV SPECTROPHOTOMETRIC METHODS

Selection of solvent

The solubility of drugs was determined in a variety of non polar to polar solvents as per I.P. specification**.** The common solvents were found to be as methanol and water for the analysis of Desloratadine and Ambroxol hydrochloride for the proposed method.

Preparation of standard stock solution

An accurately weighed quantity of AMB (40 mg) and DES (20 mg) were transferred into a separate 10 ml volumetric flask and methanol was added to both volumetric flasks. Volume was adjusted up to the mark with methanol for first dilution and further diluted with water to obtain the concentration of 10 $\mu g\ mL^{-1.}$

Selection of analytical wavelength

The selection of wavelength for the estimation of DES and AMB was done by preparing a suitable diluted stock solution containing 10 μgmL^{-1} solutions. The stock solutions were scanned between the wavelength ranges from 200 - 400 nm by using water as blank and the spectrum was recorded. For the Absorption ratio method, the absorbance's are measured at two wavelengths one being the λ max of one of the component Ambroxol hydrochloride $\lambda_{2,}$ 244 nm and the other being a equal absorptive of two component λ_1 Desloratadine 288 nm an isobestic point**.** The zero order spectra obtained and it was derivatised to first order spectrum. The values of amplitudes were measured for Ambroxol hydrochloride at 320 nm (zero crossing point for DES) and 277 nm measured for Desloratadine (zero crossing point for AMB) respectively**.**

Linearity Characteristics

For an Absorption ratio method, appropriate aliquots of stock solution of DES (1-5 ml of 5 µg/ml) and AMB (1-5 ml of 80 µg/ml) were transferred into a 10 ml volumetric flasks to get the concentration of 0.5 – 2.5 µg/ml, 8- 40 µg/ml were made up to the volume with water and measured the absorbance's at 288 nm and 244 nm. For Derivative method, aliquots of stock solution of AMB (1-5 ml of 750 µg/ml) and DES (1-5 ml of 50 µg/ml) were transferred into 10 ml volumetric flasks to get the concentration of 75 – 375 µg/ml, 5- 25 µg/ml were made up to the volume with water. The zero order spectra were derivatized to first order derivative spectra with the wavelengths 320 nm, 277 nm (zero crossing points for DES and AMB) respectively**.**

Analysis of sample formulation

Twenty tablets (DYL - AX) were weighed accurately and made into a fine powder. A quantity of tablet powder equivalent to 75 mg of Ambroxol hydrochloride was weighed and transferred into a 50 ml volumetric flask, added a minimum quantity of methanol to dissolve the substance by using ultra sonication for 15 minutes, and made up the volume to 50 ml in a volumetric flask. Then the content was filtered through Whatmann filter paper No. 41. From the cleared solution, 1 ml was taken and made up to 100 ml with water to obtain 15 µg mL^{-1} of AMB which contains 1 µg mL^{-1} of DES theoretically. The absorbance measurements were made 6 times for the absorption ratio method. For Derivative method, from the above stock solution, 5 ml was taken and made up to 100 ml with water to obtain 75 µg mL^{-1} of AMB which contains 5 µg mL^{-1} of DES theoretically by derivatising the zero order spectra into first order derivative spectra at 320 nm and 277 nm respectively.

Recovery studies

In order to ensure the reliability and suitability of the proposed method, recovery studies were carried out. It was done by mixing known quantity of standard drug with formulation sample and the content were Pre analyzed by the proposed method. To a quantity equivalent to 75 mg of AMB and standard drugs DES and AMB were added at 80% 100% and 120% levels. The analyte was extracted, diluted and re analyzed as per the formulation procedure. Absorbances were noted at respective wavelength. Recovery studies were repeated for three times and the results are shown.

VALIDATION OF THE DEVELOPED METHODS

Validation of the developed method was carried out as per ICH Guidelines. Parameters such as Linearity, Accuracy, Precision, Specificity, Robustness, LODs and LOQs were taken up as tests for method validation.

Linearity

For Quantitative analysis of DES and AMB, linearity curve was plotted. Linearity range of DES and AMB was established in the concentration range of 0.5- 2.5 µg/ml and 8-40 µg/ml for Absorption Ratio method respectively. For Derivative method the concentration ranges of 5 - 25 µg/ml and 75 - 375 µg/ml was performed.

All validated procedures was followed as per first method

4.2.5. DOXOFYLLINE AND SALBUTAMOL SULPHATE

Pharmaceutically pure sample of Doxofylline and Salbutamol sulphate were generously gifted by Himalayan Pharmaceuticals Pvt. Ltd, Himachal Pradesh.

Combination product DOXORIL PLUS containing 400 mg Doxofylline and 4 mg Salbutamol sulphate. The tablet dosage was purchased from a local Pharmacy.

The methods employed for simultaneous estimation of Doxofylline and Salbutamol sulphate in combination is

4.2.5.1 UV Spectrophotometric method

- Simultaneous equation method
- Area under curve method
- Derivative spectrophotometric method

Selection of solvent

The solubility of drugs was determined in a variety of solvents as per Indian Pharmacopoeial standards. Solubility was carried out from non polar solvents to polar solvents. The common solvent was found to be distilled water for the analysis of Doxofylline and Salbutamol sulphate for proposed method.

Preparation of standard stock solution

Accurately weighed drug samples of both DOX and SAL (20 mg each) were transferred into a suitable standard volumetric flask separately, dissolved and diluted to mark with distilled water. Both the drug in solutions was diluted so as to get 10 µg/ml. These solutions were scanned in the UV region of 200 - 400 nm in 1cm cell against distilled water as blank and the overlaid spectra was recorded.

Selection of wavelengths for estimation and stability studies

From the overlaid spectra of DOX (10 µg/ml) and SAL (10 µg/ml) in distilled water, wavelengths 274 nm (λ max of DOX) and 224 nm (λ max of SAL) were selected for the formation of Simultaneous equation method. For the Area under curve method, the wavelength selected were 220 nm – 230 nm for DOX and 270 nm – 280 nm for SAL. For Derivative Spectroscopic method, the zero order spectra was derivatised to second order spectra in that 233 nm was selected for the estimation of DOX which is zero crossing for SAL and 229 nm was selected for the estimation of SAL which is zero crossing for DOX.

Preparation of calibration graph

From the primary stock solution, aliquots were drawn and suitably diluted so as to get the final concentration range of 5-30 µg/ml of DOX and 5-30 µg/ml of SAL. Absorbances of these solutions were recorded in the respective wavelengths. For derivative method, concentration ranges from 10-60 µg/ml of DOX and 10-60 µg/ml of SAL respectively.

Analysis of tablet formulation (Standard addition method)

Twenty tablets (DOXORIL PLUS) were weighed and average weight was found. The tablets were triturated to a fine powder. An accurately weighed quantity of powder equivalent to 25 mg of DOX was transferred into a 25 ml volumetric flask, then added 24.75 mg of Salbutamol sulphate raw material and sufficient quantity of distilled water was added and the solution was sonicated for 15 minutes and diluted to the mark with distilled water. It was filtered through Whatmann filter paper No. 41, filtrate was suitably diluted to get final concentration of 15 µg/ml of DOX and 15 µg/ml of SAL with distilled water. For derivative method, the filtrate was diluted to get the expected concentration 30

µg/ml of DOX and 30 µg/ml of SAL with distilled water. The absorbance of sample solution was measured six times at all selected wavelengths for all the methods.

Recovery studies

The accuracy of the proposed methods were checked by recovery studies, by addition of standard drug solution to pre analyzed sample solution at three different concentration levels (80 %, 100 % and 120%) within the range of linearity for both the drugs. The basic concentration level of sample solution selected for spiking of the drug standard solution was 15 µg/mL of DOX and 15 µg/mL of SAL for all the methods.

Validation of developed method

The methods were validated with respects to linearity, LOD (Limit of Detection), LOQ (Limit of Quantitation), Precision, Accuracy and Ruggedness

Linearity

Linearity was checked by diluting standard stock solution at five different concentrations. DOX was linear with the concentration range of 5-30 µg/ml and SAL showed linearity in the range of 5-30 µg/ml and calibration curves [n=5] were plotted between concentration and absorbance of drugs. Optical parameters were calculated.

All validated procedures were followed as per first method.

RESULTS & DISCUSSION

5. RESULTS AND DISCUSSION

The simultaneous estimation of two drugs in a formulation has more advantages such as accurate, less use of reagent and less time requirement for the simultaneous estimation rather than individual estimation of two drugs. Novel, simple, precise and accurate analytical techniques were developed for the following combinations and to validate the methods according to ICH guidelines and applying the same for its estimation in marketed formulation. The methods includes

1. UV spectroscopic method
2. RP-HPLC method
3. HPTLC method

5.1 TOLPERISONE HYDROCHLORIDE AND PARACETAMOL

5.1.1 UV SPECTROSCOPIC METHOD

5.1.1.1 SIMULTANEOUS EQUATION METHOD

The identification of Tolperisone Hydrochloride and Paracetamol were confirmed by melting point and IR spectral studies (Figures 1-2). The solubility of Tolperisone Hydrochloride and Paracetamol were determined in variety of solvents as per Indian Pharmacopeial standards. Solubility was carried out in non – polar to polar solvents.

Distilled water was selected as a common solvent. The solubility profile of Tolperisone Hydrochloride and Paracetamol are given in the Table 1 and 2 respectively.

The sample solution of 10 µg/ml of Tolperisone Hydrochloride and Paracetamol were prepared individually and the solutions were scanned between 200 – 400 nm by using water as blank as shown in Figure 3. From the overlaid spectra by observing the spectral characteristics λ max of TPE at 261 nm and λ max of PCL at 243 nm was selected for simultaneous equation method.

The stability of the drug solution was observed at different time intervals. Paracetamol was stable for 6 hours and Tolperisone Hydrochloride was stable for 5 hours. From the aliquots of stock solution of TPE and PCL, concentration (0.5-2.5 µg/ml, 3-15 µg/ml) were prepared. The calibration curve was plotted with absorbance versus concentration for the two drugs. The optical characteristics such as correlation coefficient slope, intercept, LOD and LOQ were calculated and regression equation was constructed.

The correlation coefficient was found to be 0.999846 for TPE at 261 nm and 0.9996385 for TPE at 243 nm. At 261 nm the LOD and LOQ were found to be 0.3872 µg/ml and 1.173497 µg/ml for Paracetamol. At 243 nm the LOD and LOQ were found to be 0.02261914 µg/ ml and 0.0685427µg/ml for Paracetamol. At 243 nm, the LOD and LOQ were found to be 0.8457855 µg/ml and 2.56299 µg/ml for Tolperisone, 0.007044237 µg/ml and 0.21346143 µg/ml for TPE.

The correlation coefficient values at all the selected wavelengths are found to be above 0.999. Hence the selected concentrations are linear and obeyed Beer's law. The calibration graphs for Tolperisone Hydrochloride at 243 nm, 261 nm are shown in Figure 5 and 6 respectively. The calibration graphs for Paracetamol at 243 nm and 261 nm are shown in Figure 7 and 8.

The optical characteristics at 243 nm, 261 nm are shown in Tables 3 and 4 respectively. The developed method was applied for the analysis of synthetic mixture to find out the developed method was correct or not. The % average of synthetic mixture was found to be 100.104 for TPE and for PCL 100.102 (Table-5). The amount found was good agreement with the expected concentration. Hence it was planned to apply for the analysis of formulation.

The percentage purity of drugs in the formulation was found to be 100.0183 ± 0.04167 for Paracetamol, 99.558 ± 0.48602 for Tolperisone Hydrochloride. The precision of the method was confirmed by the repeated analysis of the formulation for six times. The percentage RSD was calculated. The percentage RSD was found to be 0.41666 for PCL and 0.488219 for TPE respectively. The low % RSD values suggest that the method has good precision. The results are shown in Table 6.

Further, precision of the method was confirmed by Intraday and Inter day analysis. Intraday and Interday analysis of formulation was done for three times on same day and one time on three consecutive days. The percentage RSD for the Intraday and Interday precision of TPE was found to be 0.5030 and for PCL 0.1969 (Table 7). The low % RSD values suggest that the precision of the method was further confirmed.

The Ruggedness study was performed with different instruments and the results were shown in the Table 8.The accuracy of the method was confirmed by recovery studies. The percentage recovery was found to be in the range of 100.1 ± 1.2230 for Tolperisone, 100.06 ± 0.08144 for Paracetamol.

The percentage RSD was found to be 1.2218 for TPE and 0.08139 for PCL. The low percentage RSD indicated that there was no interference due to excipients used in

formulation. Hence, the accuracy of the method was confirmed. The data for recovery studies are given in Table 9.

5.1.1.2 ABSORPTION RATIO METHOD

A simple, accurate, rapid precise Absorption Ratio method was developed and validated. Distilled water was chosen as a common solvent for the estimation of Tolperisone Hydrochloride and Paracetamol. The sample solution of 10 µg/ml Tolperisone Hydrochloride and Paracetamol were prepared individually and the solutions were scanned between 200 – 400 nm by using water as blank and shown in Figure 3.

From the overlaid spectra by observing the spectral characteristics the absorbances are measured at two wavelengths, one being the λ max of one of the components Paracetamol λ2 243 nm and the other being an equal absorptivity of two component λ_1 Tolperisone Hydrochloride 254 nm an iso-absorptive point. Appropriate aliquot of serial dilution was made in the concentration range from 0.5-2.5 µg/ml, 3-15 µg/ml. By observing, concentration was proportional to absorbance and it obeys Beer's law. The optical characteristics for Tolperisone Hydrochloride are listed in the Table 10. Calibration graphs were given in the Figure 9&10.

The correlation co-efficient of Paracetamol was found to be 0.99998 at 254 nm and 0.99952 at 243 nm (Table 11). At 254 nm, the LOD and LOQ were found to be 0.0020741 µg/ ml and 0.006285 µg/ ml for Tolperisone, at 243 nm 0.113188 µg/ ml and 0.34299 µg/ ml for PCL. The developed method was applied for the analysis of synthetic mixture to find out the developed method was correct or not.

The % average of synthetic mixture was found to be 99.99 for TPE and for PCL 100.266 (Table-12). The amount found was good agreement with the expected concentration. Hence it was planned to apply for the analysis of formulation.

The percentage purity of drugs in the formulation was found to be 98.97 ± 0.87395 and 99.88 ± 0.16940 for Tolperisone Hydrochloride and Paracetamol. The precision of the method was confirmed by the repeated analysis of the formulation for six times. The percentage RSD was calculated. The percentage RSD of Tolperisone Hydrochloride and Paracetamol were found to be 0.88305 and 0.16959 respectively.

The low RSD values suggest that the method has good precision. The results are shown in Table 13. The precision was confirmed by Intraday and Inter day analysis. Intraday and Interday analysis of formulation was done on three times on same day and one time on three consecutive days.

The percentage RSD for the Intraday and Inter day precision was found to be 0.1687 for TPE and 0.4588 for PCL. The low % RSD values suggest that the precision of the method was further confirmed (Table 14). The ruggedness study was performed by different analyst and different instrument. The % RSD of analyst was found to be 0.1232 and 0.6032, listed in the Table 15.

The Accuracy was confirmed by recovery studies. The percentage recovery was found to be in the range of 99.71 ± 0.23692 for Tolperisone Hydrochloride, 99.94633 ± 0.10084 for Paracetamol are listed in the Table 16.

.

5.1.1.3 AREA UNDER CURVE METHOD

A simple, accurate, rapid, precise Area under curve method was developed and validated. Distilled water was chosen as a common solvent for the estimation of Tolperisone Hydrochloride and Paracetamol. The sample solution of 10 µg/ml of Tolperisone Hydrochloride and Paracetamol were prepared individually and the solutions were scanned between 200 – 400 nm by using water as blank as shown in Figure 3.

From the overlaid spectra by observing the spectral characteristics the absorbances are measured at 253 nm - 269 nm for TPE and 274 nm – 284 nm for PCL. Aliquots of serial dilution were made in the concentration range from 0.5-2.5 µg/ml, 3-15 µg/ml. By observing, concentration was proportional to absorbance and it obeys Beer's law. The optical characters are listed in the Table 17 and 18. Calibration graph for TPE were shown in the Figure 13 & 14 and for PCL, Figure 15 & 16 respectively.

The correlation co-efficient of Paracetamol was found to be 0.99965 and for Tolperisone Hydrochloride 0.99921 at 253 nm – 269 nm. At 274 nm - 284nm the LOD and LOQ were 0.143268 and 0.487699 PCL. The developed method was applied for the analysis of synthetic mixture to find out the developed method was correct or not. The % average of synthetic mixture was found to be 100.0536 for TPE and for PCL 99.814 (Table -19). The amount found was good with the expected concentration. Hence it was planned to apply for the analysis of formulation.

The percentage purity of drugs in the formulation was found to be 99.83 ± 0.31864 and 99.8466 ± 0.22429 for Tolperisone Hydrochloride and Paracetamol. The precision of the method was confirmed by the repeated analysis of the formulation for six times. The percentage RSD was calculated (Table-20). The percentage RSD of Tolperisone

Hydrochloride and Paracetamol were found to be 0.3190 and 0.22463 respectively. The low % RSD values suggest that the method has good precision.

The precision was confirmed by Intraday and Inter day analysis. Intraday and Inter day analysis of formulation was done on three times on the same day and one time on three consecutive days. The percentage RSD for the Intraday and Inter day precision was found to be 0.40193 and 0.07098(Table -21). The low % RSD values suggest that the precision of the method was further confirmed. The Ruggedness study was performed with different instruments and the % RSD was found to be 0.3604, 0.41766 for TPE (Table -22).

The Accuracy was confirmed by recovery studies. The percentage recovery was found to be in the range of 99.688 ± 0.7999 for Tolperisone Hydrochloride, 99.91 ± 0.22108 for Paracetamol. The % RSD values were found to be 0.79908 and 0.22123 (Table 23) respectively.

5.1.1.4 DERIVATIVE SPECTROSCOPIC METHOD

Derivative spectrophotometer involves the conversion of a normal spectrum to its first, second or higher derivative spectrum. Distilled water was chosen as a common solvent for the estimation of Tolperisone Hydrochloride and Paracetamol.

The sample solution of 10 µg/ml of Tolperisone Hydrochloride and Paracetamol were prepared individually and the solutions were scanned between 200 – 400 nm by using water as blank as shown in Figure 4. A normal spectrum was derivatised to first order in which 243 nm was selected for TPE which is zero crossing point for PCL and 261 nm was selected for PCL which is zero crossing point for TPE.

Aliquots of serial dilution were made in the concentration range from 1-5 µg/ml, 6-30 µg/ml. The correlation co-efficient for Tolperisone Hydrochloride was found to be 0.99912 at 243 nm and 0.99968 at 261 nm. The calibration graphs were shown in the Figure 11 & 12. At 261 nm the LOD and LOQ were 0.984396 and 2.983018 (Table - 24). Synthetic mixture was performed and the results are given in the Table - 25. The percentage purity of drugs in the formulation was found to be 100.026 ± 0.06653 and 99.7542 ± 0.3070 for Tolperisone Hydrochloride and Paracetamol (Table – 26).

The precision of the method was confirmed by the repeated analysis of the formulation for six times. The percentage RSD was calculated. The percentage RSD of Tolperisone Hydrochloride and Paracetamol were found to be 0.06651 and 0.3077 respectively. The low RSD values suggest that the method has good precision. The precision was confirmed by Intraday and Inter day analysis. Intraday and Inter day analysis of formulation was done on three times on same day and one time on three consecutive days.

The percentage RSD for the Intraday and Inter day precision was found to be 0.2670 and 0.2137 for TPE. The low RSD values suggest that the precision of the method was further confirmed. The Ruggedness study was performed and listed in the Table 28. The Accuracy was confirmed by recovery studies.

The percentage recovery was found to be in the range of 100.013 ± 0.04728 for Tolperisone Hydrochloride, 100.048 ± 0.4780 for Paracetamol (Table – 29). The % RSD values were found to be 0.0425 and 0.4779 respectively.

5.1.2. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

In RP-HPLC method, the conditions were optimized to obtain an adequate separation of eluted compounds. Initially various mobile phase composition were attempted to elute title ingredient. Mobile phase and flow rate selection was based on peak parameters (height capacity, theoretical plates, tailing or symmetry factor) run time and resolution.

The system with mobile phase containing Acetonitrile: Methanol (50:50 % v/v) was initially performed and chromatogram was recorded. Finally the mobile phase consists of Methanol: Acetonitrile with 0.1 ml of 0.1% triethylamine with the ratio of 60: 40 % v/v was tried. After calculating all system suitability parameters Methanol: Acetonitrile with 0.1 ml of 0.1% Triethylamine in the ratio of 60: 40 % v/v at flow rate of 1.0 ml/ min was selected.

The retention time for Tolperisone Hydrochloride and Paracetamol was found to be 2.915± 0.1 min and 4.637 ± 0.1 min respectively and with a resolution of 9.087 which is better resolution.

According to ICH Guidelines, system suitability tests are integral part of chromatographic method. They are used to verify the reproducibility of chromatographic method. To ascertain the methods, effective system suitability tests are carried out on freshly prepared stock solutions of Tolperisone Hydrochloride and Paracetamol were prepared by using methanol (for first dilution only) and mobile phase of various concentrations were prepared in the range of 2-10 µg/ ml of Tolperisone Hydrochloride and 4-20 µg/ ml of Paracetamol respectively. 20 µl of each solution were injected individually and the chromatograms were recorded at 254 nm. The chromatograms are shown Figures 17-21.

The calibration curve was plotted using concentration against peak area. The procedure was repeated for three times. The correlation co - efficient value was around 0.999 for two drugs. It indicates that the concentrations of Tolperisone Hydrochloride and Paracetamol had good linearity. The calibration graphs are shown in Figures 22 and 23. The optical characteristics of Tolperisone Hydrochloride were shown in the Table 30.

The tablet dosage form MYO-MR PLUS was selected for the analysis. The drug Tolperisone Hydrochloride and Paracetamol are in ratio 1:2 in the formulation. The concentration of 12 μg/ml of Paracetamol which is also containing 6 μg/ml of Tolperisone Hydrochloride in the mobile phase was prepared. 20 μl of each solution was injected and chromatograms were recorded. The percentage purity was found to be 99.83 ± 0.28304 and 99.93 ± 0.00460 for Tolperisone Hydrochloride and Paracetamol respectively.

The precision of the method was confirmed by repeatability of formulation for six times and the chromatograms are shown in Figures 24 – 26. The percentage RSD was found to be 0.28354 and 0.00460 for Tolperisone Hydrochloride and Paracetamol respectively. It indicates that the method has good precision. The data for the analysis of formulation is shown in Table 31. The system suitability parameters were listed in the Table-32.

The precision was confirmed by Intraday and Inter day analysis. Intraday and Inter day analysis of formulation was done on three times on same day and one time on three consecutive days.The percentage RSD for the Intraday and Inter day precision was found to be 0.43798 and 0.69691 for TPE were shown in the Table-33. The low RSD values suggest that the precision of the method was further confirmed.

The accuracy of the method was performed by recovery studies. To the pre analyzed formulation, a known quantity of Tolperisone Hydrochloride and Paracetamol raw material solutions were added at different levels and injected the solutions.

The chromatograms were recorded as shown in the Figure 27- 29. The percentage recovery was found to be in the range between 99.51 ± 0.24131 and 99.50 ± 0.33866. The % RSD was found to be 0.24248 for Tolperisone Hydrochloride and 0.33453 for Paracetamol. The low % RSD values for recovery indicated that the method was found to be accurate. The values are given in the Table 34.

The high percentage recovery revealed that no interference produced due to the excipients used in formulation. Therefore, the developed method was found to accurate.

5.1.3 HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

An effort was made to develop a simple, precise and accurate method for the simultaneous estimation of Tolperisone Hydrochloride and Paracetamol in bulk and in Pharmaceutical dosage form by HPTLC method. The initial separation was based up on the solubility of drugs. The different mobile phase were tried to get the better resolution.

The different mixtures of the mobile phase tried were Chloroform : Toluene : Methanol : Glacial Acetic Acid, Chloroform : water: : Acetic Acid, Chloroform : Toluene : Methanol : Glacial Acetic Acid and Benzene : Toluene : Methanol : Glacial Acetic Acid with different ratios. After various trials, Chloroform: acetic acid: water (6:2:2 v/v/v) was selected. With the above selected mobile phase, the UV spectra of all the drugs were recorded and overlaid.

From the overlain spectra, at 264 nm both the drugs showed marked absorbance. The Rf value for both the drugs was found to be 0.9634 and 0.7926 respectively. The linearity range was fixed as 20 - 100 ng/µl for Tolperisone Hydrochloride and 40 – 200 ng/µl for Paracetamol in methanol and shown in Figures 30-34. The calibration graph was recorded using peak area and concentration and these are shown in Figures 35 – 36.

The correlation coefficients were found to be 0.9999, 0.9997 for Tolperisone Hydrochloride and Paracetamol respectively. The optical characteristics such as the Correlation coefficient, Slope, Intercept, LOD and LOQ and were calculated and shown in Table 35. The correlation coefficient values indicated that the selected concentration was linear.

The tablet dosage form MYO-MR PLUS was selected for the analysis. The chromatogram for the analysis of formulation was shown in Figures 37-39. The percentage purity of Tolperisone Hydrochloride and Paracetamol were found to be 99.883 ± 0.20925, 99.978 ± 0.06645. The results of analysis are shown in Table 36. Precision of the method was confirmed by repeated analysis of formulation for six times. The percentage RSD values were found to be 0.20949, 0.06647 for Tolperisone Hydrochloride and Paracetamol respectively**.** Further the precision of the method was confirmed by intraday and inter day studies.The results were listed in the Table-37.

The accuracy of the method was confirmed by the recovery studies. To the pre-analyzed formulation, a known quantity of raw material was added and the percentage recovery was calculated. The percentage of raw material added was 80%, 100% and 120% for both drugs.

The chromatograms for the recovery analysis are shown in Figures 40-42.The percentage recovery was found to be in the range of 99.50 ± 0.47056 and 99.51 ± 0.24131. The percentage RSD values were found to be 0.24248 and 0.472852 for Tolperisone Hydrochloride and Paracetamol respectively.

The low percentage RSD value indicates that there was no interference due to the excipients used in formulation during the analysis. The data of recovery analysis are listed in Table 38.

5.2 SITAGLIPTIN PHOSPHATE AND SIMVASTATIN

5.2.1 DERIVATIVE SPECTROSCOPY METHOD

The identification of Sitagliptin phosphate and Simvastatin were confirmed by melting point and IR spectral studies (Figures 43 & 44). The solubility of Sitagliptin phosphate and Simvastatin were determined in variety of solvents as per Indian Pharmacopeial standards. (Table 39 & 40) Solubility was carried out in non – polar to polar solvents.

Methanol was chosen as a common solvent for the estimation of Sitagliptin phosphate and Simvastatin. The difference between spectra of standard solutions of Sitagliptin phosphate and Simvastatin versus their solvent blanks was recorded in the range of 200-380 nm. The overlaid spectra of SITA and SIM were recorded as shown in Figure 45.

The UV spectrum of SIM exhibited three well defined peaks at 233 nm, 238 nm and 247 nm and virtually no absorbance above 259 nm where SITA exhibited a broad peak covering 268 nm as broad peak. Hence multi component analytical method may not be possible for the simultaneous estimation method. Hence alternative method for SIM and SITA is by using derivative spectroscopic method.

The first order derivative spectra of the standard solutions of each drug and those containing mixtures of both drugs were obtained in the same range of wavelength (200-380 nm) against blanks as shown in Figure 46. From the spectrum, 277 nm and 238 nm were selected for the estimation of SITA and SIM respectively without any interference. Experimental procedures describes calibration curve, assay of tablets, recovery studies, precision studies, LODs & LOQs.

A critical evaluation of proposed method was performed statistical analysis of data where slope, intercept and correlation coefficient was studied and shown in the Table 41. Beer's law obeys in the concentration range of 10-50 µg/ml, 4-20 µg/ml for each drug and correlation coefficient was 0.999301 for SITA and 0.999131 for SIM. The plotted graphs are shown in the Figure 47 and 48 respectively.The results of Synthetic mixtures are listed in the Table – 42. The proposed method was also evaluated by assay of commercially available tablets containing SIM & SITA (n=6). The results are shown in Table 43.

The percentage purity of drugs in the formulation was found to be 99.97 ± 0.6003 for SIM and 99.745 ± 0.79455 for SITA. The low % RSD value indicates that the method has good precision. Further the precision of the method was confirmed by Intraday and Interday analysis. The analysis of formulation was carried out for three times in the same day and one time in the three consecutive days. The results of analysis are shown in Table 44. Hence 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 source of reagents and so on. The low % RSD values indicate that the developed method was more rugged. The results are shown in Table 45.

The accuracy of the method was performed by recovery studies. To the preanalyzed formulation, a known quantity of SIM and SITA raw material solutions were added at three levels (80%, 100%, and 120%).The absorbances of the solution were measured and the % recovery was calculated. The % recovery assay was found to be 100.273 for SIM and 99.67 for SITA. The % RSD value was found to be 0.52395 for SIM and 0.4181533 for SITA. The accuracy and reproducibility is evident from the data and are shown in Table 46.

5.2.2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Novel, simple, accurate, rapid and precise method was developed and validated for the estimation of Sitagliptin Phosphate and Simvastatin in pure form and in tablet dosage form by an isocratic RP-HPLC method.

The solution of 10 μgmL^{-1} SITA and SIM were prepared in mobile phase using Acetonitrile: Methanol (50:50 % v/v) and the solutions were scanned in the range of 200 nm - 400 nm. It was found that the two drugs have marked absorbance at 251 nm and can be effectively used for estimation of two drugs without interference. Therefore 251 nm was selected as detection wavelength for the estimation of two drugs by RP-HPLC method with an isocratic elution technique and it was found that the two drugs are stable for approximately two hour.

In RP-HPLC method, the conditions were optimized to obtain an adequate separation of eluted compounds. Initially various mobile phase composition were attempted to elute title ingredient. Finally the mobile phase consists of Methanol: Acetonitrile with 0.1 ml of 0.1% triethylamine with the ratio of 60:40% v/v was tried.

After calculating all system suitability parameters Methanol: Acetonitrile with 0.1 ml of 0.1% Triethylamine in the ratio of 60: 40 % v/v at flow rate of 1.0 ml/ min was selected. The retention time for SITA and SIM was found to be 4.03 and 6.8 minutes respectively.

According to ICH Guidelines, system suitability tests are integral part of chromatographic method. The system suitability parameters for optimized chromatogram are shown in Table 51. They are used to verify the reproducibility of chromatographic method.

To ascertain methods, effective system suitability tests are carried out on freshly prepared stock solutions of SITA and SIM were prepared by using methanol (for first dilution only) as mobile phase at various concentrations were prepared in the range of 100 - 500 µg/ml of SITA and 40 - 200 µg/ml of SIM respectively. 20 µl of each solution were injected individually and the chromatograms were recorded at 251 nm. The chromatograms are shown Figures 49 – 53.

The calibration curve was plotted using concentration against peak area. The procedure was repeated for three times. The correlation co - efficient value was around 0.999 for two drugs. It indicates that the concentrations of SITA and SIM had good linearity. The calibration graphs are shown in Figures 54 and 55. The optical characteristics are shown in Table 47.

The tablet dosage form Juvisync was selected for the analysis. The concentration of 300 μg/ ml of SITA which is also containing 120 μg/ ml of SIM in the mobile phase was prepared. 20 μl of each solution was injected and chromatograms were recorded.

The percentage purity was found to be 100.108 ± 0.410614 and 99.58 ± 0.85773 for SIM and SITA respectively. The precision of the method was confirmed by repeatability of formulation for six times and the chromatograms are shown in Figures 56 – 58.

The percentage RSD was found to be 0.410119 and 0.861322 for SIM & SITA respectively. It indicates that the method has good precision. The data for the analysis of formulation is shown in Table 48. Further the precision of the method was confirmed by Intraday and Interday analysis. The analysis of formulation was carried out for three times in the same day and one time in the three consecutive days. The results of analysis are shown in Table 49.

The accuracy of the method was performed by recovery studies (Figure-59-61). The percentage recovery was found to be in the range between 100.105 ± 0.607207 for SITA and 100.086 ± 0.75719 for SIM. The % RSD was found to be 0.60659 for SITA and 0.075653 for SIM. The low % RSD values for recovery indicated that the method was found to be accurate. The values are given in the Table 50. The high percentage recovery revealed that no interference produced due to the excipients used in formulation. Therefore, the developed method was found to accurate.

5.2.3 HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

An effort was made to develop a simple, precise and accurate method for the simultaneous estimation of Sitagliptin Phosphate and Simvastatin bulk and in Pharmaceutical dosage form by HPTLC method.

The initial separation was based upon the solubility of drugs, the different mobile phase were tried to get the better resolution. The different mixtures of the mobile phase tried were Chloroform : Toluene : Methanol : Glacial Acetic Acid, Chloroform : water : Acetic Acid, Chloroform : Toluene : Methanol : Glacial Acetic Acid and Benzene : Toluene : Methanol : Glacial Acetic Acid with different ratios. After various trials Toluene: methanol: acetic acid: (5:4:1 v/v/v) was selected. With the above selected mobile phase the UV spectra of all the drugs were recorded and overlaid. From the overlaid spectra, at 255 nm both the drugs showed marked absorbance.

The Rf values for both the drugs were found to be 0.5241 for SITA and 0.7865 for SIM respectively. The linearity range was fixed as 100 – 500 ng/µl for SITA and 40 – 200 ng/µl for SIM in methanol and shown in Figures 62- 66. The calibration graph was recorded using peak area Vs concentration and these are shown in Figures 67-68. The correlation coefficients were found to be 0.99972 for SITA and 0.9997 for SIM respectively.

The optical characteristics such as the Correlation coefficient, Slope, Intercept, LOD and LOQ and were calculated and shown in Table 52. The correlation coefficient values indicated that the selected concentration was linear. The tablet dosage Juvisync was selected for the analysis. The concentration of 300 µg/ml of SITA which is also containing 120 µg/ml of SIM in the mobile phase was prepared. 1 µl spots of each solution were placed on the plates and chromatograms were developed in the twin trough chamber.

The chromatogram for the analysis of formulation was shown in Figures 69 – 71. The percentage purity of SIM were found to be 99.78 ± 0.632712 and for SITA 99.9830 ±

0.175351. The results of analysis are shown in the Table 53. Precision of the method was confirmed by repeated analysis of formulation for six times. The percentage RSD values were found to be 0.634101 for SIM and 0.175381 for SITA respectively. Intraday and Interday results were shown in the Table-54.

The accuracy of the method was confirmed by the recovery studies. The chromatograms for the recovery analysis are shown in Figures 72 – 74. The percentage recovery was found to be in the range of 99.82 ± 0.22141 for SIM and 100.11 ± 0.42461for SITA .The percentage RSD values were found to be 0.22182 for SIM and 0.424151 for SITA respectively. The low percentage RSD value indicates that there was no interference due to the excipients used in formulation during the analysis. The data of recovery analysis are listed in Table 55.

5.3. THIOCOLCHICOSIDE AND KETOPROFEN

5.3.1. DERIVATIVE SPECTROSCOPY METHOD

The identification of Thiocolchicoside and Ketoprofen were confirmed by melting point and IR spectral studies (Figures 75 & 76). The solubility of Thiocolchicoside and Ketoprofen were determined in variety of solvents as per Indian Pharmacopeial standards.

Solubility was carried out in non – polar to polar solvents as shown in Table 56 and 57. Methanol and water was chosen as a common solvent for the estimation of Thiocolchicoside and Ketoprofen.

The standard solution of 10 µg/ml Thiocolchicoside and Ketoprofen were prepared individually and the solutions were scanned between 200 – 400 nm by using water as blank. The overlaid spectra of THI and KET were recorded as shown in Figure 77. The

UV spectrum of THI and KET has similar λ max. Hence multi component analytical method may not be possible for the simultaneous estimation method.

Hence alternate method for THI and KET is by using derivative spectroscopic method. From the overlaid spectra, the zero order spectra obtained and it was derivatised to first order spectrum and shown in the Figure 78. The values of amplitudes were measured for THI at 233.5 nm (zero crossing point of KET) and 259 nm measured for KET (zero crossing point of THI) respectively. Experimental procedures describes, calibration curve, assay of tablets, recovery studies, precision studies, LODs & LOQs.

A critical evaluation of proposed method was performed, statistical analysis of data where slope, intercept, correlation coefficient was studied. Beer's law obeys in the concentration range of 4 - 24 μg/ml, 50 - 300 μg/ml for each drug and correlation coefficient was 0.999590 for THI and 0.999945 for KET and are presented in the Table 58. The plotted graphs are shown in the Figure 79 and 80 respectively.

The Synthetic mixture was performed and presented in Table 59. The proposed method was also evaluated by assay of commercially available tablets containing THI & KET (n=6) and the results were shown in the Table 60. The percentage purity of drugs in the formulation was found to be 100 ± 0.866025 for THI and 100.263 ± 0.419031 for KET. Further the precision of the method was confirmed by Intraday and Interday analysis. The analysis of formulation was carried out for three times in the same day and one time in the three consecutive days.

The % R.S.D for Intraday and Interday precision of Thiocolchicoside was found to be 0.224809, 0.30668 and for Ketoprofen was found to be 0.191018, 0.020143.The results

of analysis are shown in Table 61. Hence the precision of the method was further confirmed.

The developed method was validated for Ruggedness. It refers to the lab to multiple days which may include multiple analysts, multiple instruments and different source of reagents and so on. The low % RSD values indicate that the developed method was more rugged. The results are shown in Table 62.

The % recovery assay was found to be 100.333 for THI and 100.0338 for KET. The % RSD value was found to be 0.339066 for THI and 0.12033 for KET and results are shown in the Table 63.

5.3.2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Reverse phase chromatography was chosen because of its recommended use for ionic and moderate to non-polar compounds. Reverse phase chromatography is not only simple, convenient but also better perform is in terms of efficiency, stability and reproducibility. C_{18} column allows eluting polar compounds more quickly compare to non-polar compounds.

In addition to this, UV detector is used, which allows easy detection of the compounds in UV transparent organic solvents. Isocratic mode was chosen due to simplicity in application and robustness with respect to longer column stability. This configuration provides a large number of theoretical plate values for most separation.

The detection was carried out in the UV region and wavelength selected for detection was 300 nm in mobile phase. The mobile phase should be sufficiently transparent at the wavelength of detection i.e. minimum absorbance.

Different compositions of acetonitrile, methanol and water were tried for selection of the mobile phase. Reason to select Acetonitrile was that it is best initial choice of organic

solvent for the mobile phase. Acetonitrile - water mixture can be used with UV detection at low wavelength. Acetonitrile - water mixture also has lower viscosity, resulting in higher number of plates and lower column back pressure than methanol - water mixture. Methanol was chosen because it is next best organic solvent after acetonitrile.

Water was selected because it is best Universal solvent. It has more viscosity than methanol and acetonitrile. In studies, various mobile phases with different ratios were used. The mobile phase consists of Acetonitrile: Water (60:40 v/v) provided optimum polarity for proper migration, separation and resolution of Thiocolchicoside and Ketoprofen.

The retention time for THI and KET was found to be 3.743 ± 0.1 min and 7.903 ± 0.1 min respectively. According to ICH Guidelines, system suitability tests are integral part of chromatographic method. They are used to verify the reproducibility of chromatographic method.

To ascertain methods, effective system suitability tests are carried out on freshly prepared stock solutions of THI and KET by using methanol (for first dilution only) as mobile phase at various concentrations were prepared in the nominal range of 6.4 - 9.6 μg/ml THI and 80 - 120 μg/ml KET respectively. 20 μl of each solution were injected individually and the chromatograms were recorded at 300 nm. The chromatograms are shown in Figures 81- 85.

The calibration curve was plotted using concentration against peak area. The correlation co - efficient value was found above 0.999 for two drugs. It indicates that the concentrations of THI and KET had good linearity. The calibration graphs are shown in Figures 86 and 87. The optical characteristics are shown in Table 64.

The percentage purity was found to be 100.133 ± 0.621825 and 100.1933 ± 0.55492 for THI and KET respectively. The precision of the method was confirmed by repeatability of formulation for six times and the chromatograms are shown in Figures 88-90.

The percentage RSD was found to be 0.620997 and 0.553859 for THI & KET respectively. It indicates that the method has good precision. The data for the analysis of formulation is shown in Table 65.

The precision of the method was confirmed by Intraday and Interday analysis. The analysis of formulation was carried out for three times in the same day and one time in the three consecutive days. The % R.S.D for Intraday and Interday precision of Thiocolchicoside was found to be 0.152885, 0.13786 and for Ketoprofen was found to be 0.142282, 0.272567. The results of analysis are shown in Table 66.

The accuracy of the method was performed by recovery studies (Figures 91-93).The percentage recovery was found to be in the range between 100.45 ± 0.526996 for THI and 100.094 ± 0.111369 for KET. The % RSD was found to be 0.524635 for THI and 0.111264 for KET. The low % RSD values for recovery indicates that this method was found to be accurate. The values are given in the Table 67. The system suitability parameters were listed in the Table-68.

The high percentage recovery revealed that no interference produced due to the excipients used in formulation. Therefore, the developed method was found to be accurate.

5.4 DESLORATADINE AND AMBROXOL HYDROCHLORIDE

The identification of Desloratadine and Ambroxol hydrochloride were confirmed by melting point and IR spectral studies (Figures 94 & 95). The solubility studies were performed and presented in Table 69 & 70.

5.4.1.1 ABSORPTION RATIO METHOD

A simple, accurate, rapid, precise Absorption Ratio method was developed and validated. Methanol and Distilled water was chosen as a common solvent for the estimation of Desloratadine and Ambroxol hydrochloride. The sample solution of 10 µg/ml Desloratadine and Ambroxol hydrochloride were prepared individually and the solutions were scanned between 200 – 400 nm by using water as blank.

From the overlaid spectra (Figure 96) by observing the spectral characteristics the absorbance's are measured at two wavelengths one being the λ max of one of the components Ambroxol hydrochloride λ_2 244 nm and the other being a equal absorptivity of two component λ_1 Desloratadine 288 nm an iso-absorptive point.

Appropriate aliquot of serial dilution was made in the concentration ranges from 0.5-2.5 µg/ml, 8-40 µg/ml. By observing, concentration was proportional to absorbance and it obeys Beer's law. The optical characters were listed in the Table 71 and 72. The plotted graph of DES & AMB are shown in the Figure 98-101. The correlation co-efficient of Desloratadine was found to be 0.9996279 at 244 nm and 0.99992637 at 288 nm. At 244 nm, the LOD and LOQ were found to be 0.0054673 µg/ml and 0.0165678 µg/ml for Desloratadine, at 288 nm 0.34933 µg/ml and 1.05860 µg/ml for Ambroxol hydrochloride.

The developed method was applied for the analysis of synthetic mixture to find out the developed method was correct or not. The amount of Desloratadine and Ambroxol hydrochloride were found to be in the range of 100.022% and 100.238% (Table-73). The amount found was good agreement with the expected concentration. Hence it was planned to apply for the analysis of formulation.

The percentage purity of drugs in the formulation was found to be 100.218 ± 0.541125 for Desloratadine and 99.97 ± 0.31686 for Ambroxol hydrochloride and shown in the Table 74. The precision of the method was confirmed by the repeated analysis of the formulation for six times. The percentage RSD was calculated. The percentage RSD of Desloratadine and Ambroxol hydrochloride were found to be 0.539946 and 0.316955 respectively.

The low RSD values suggest that the method has good precision. Intraday and Interday analysis of formulation was done on three times on same day and one time on three consecutive days. The percentage RSD for the Intraday and Inter day precision was found to be 0.212388 and 0.550644 for DES and for AMB 0.09665, 0.33541 respectively. The low % RSD values suggest that the precision of the method was further confirmed.

The ruggedness of the method was confirmed by performing the analysis with the different analysts and different instruments. The percentage RSD values for different analysts were found to be 0.501354 and 0.14142 for Desloratadine and for Ambroxol hydrochloride 0.80284 and 0.41766 respectively. The percentage RSD values for different instruments were found to be 0.14132 and 0.001768 for Desloratadine and for Ambroxol hydrochloride 0.22076 and 0.09448 (Table 76).

The Accuracy was confirmed by recovery studies. The percentage recovery was found to be in the range of 101.02 ± 0.61650 for DES and 100.287 ± 0.521063 for AMB and shown in the Table 77.

5.4.1.2 DERIVATIVE SPECTROSCOPY METHOD

A simple, accurate, rapid precise Derivative method was developed and validated. Methanol and water was chosen as a common solvent.The standard solutions of AMB and DES were scanned separately in the UV range, and zero-order spectra thus obtained was then processed to obtain first-derivative spectra (Figure 97). Data were recorded at an interval of 0.1 nm. The two derivative spectra showed significance absorbance at 320 nm (ZCP of DES) for AMB and 277 nm (ZCP of AMB) for DES. First order derivative absorbance (D1) was recorded at 320 nm for AMB and 277 nm for DES.

First order derivative spectra give good quantitative determination of both the drugs at their respective wavelength without any interference from the other drug in their combined dosage formulations. Linear correlation was obtained for DES in the concentration ranges of 5 – 25 µg/ml and AMB 75 - 375 µg/ml respectively. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression (Table 78). The LOD of DES at 277 nm was found to be 0.1446132 and for AMB at 320 nm were 50.141566.The calibration graphs was shown in the Figure 102 & 103 respectively.

From the Analysis of synthetic mixture the % average was found to be 100.52% for DES and for AMB 99.914% was shown in the Table -79. The percentage purity of drugs in the formulation was found to be 100.5 ± 0.532917 and 99.82 ± 1.0461 for Desloratadine and Ambroxol hydrochloride were shown in the Table 80. The precision of the method was confirmed by the repeated analysis of the formulation for six times

The percentage RSD was calculated. The percentage RSD of Desloratadine and Ambroxol hydrochloride were found to be 0.530265 and 1.0479 respectively. The relative standard deviation (less than 2 %) indicates that the proposed method is repeatable. The RSD values of interday was 0.50742 and intraday was 0.362073 for DES and for AMB interday was 0.55459 and intraday 0.16812 respectively (Table 81).

These data show that proposed method is sensitive for the determination of AMB and DES. The ruggedness of the method was confirmed by performing the analysis with the different analysts and different instruments. The percentage RSD values for different analysts were found to be 1.8396 and 1.5954 for Desloratadine and for Ambroxol hydrochloride 1.7256 and 1.8602 respectively. The percentage RSD values for different instruments were found to be 0.6684 and 0.4463 for Desloratadine and for Ambroxol hydrochloride 1.9820 and 1.0783 respectively (Table 82).

The recovery experiment was performed by the standard addition method. The mean % recoveries were 99.46 ± 1.0061 and 100.013 ± 0.2369 for DES and AMB respectively (Table 83).

The results of recovery studies indicate that the proposed method is accurate. The proposed validated method was successfully applied to determine AMB and DES in their combined dosage form. This is also a cost effective method. The additives usually present in the pharmaceutical formulation of the assayed sample did not interfere with determination of AMB and DES. The method can be used for the routine analysis of the AMB and DES in combined dosage form without any interference of excipients**.**

5.5 DOXOFYLLINE AND SALBUTAMOL SULPHATE

5.5.1.1 SIMULTANEOUS EQUATION METHOD

The identification of Doxofylline and Salbutamol Sulphate were confirmed by melting point and IR spectral studies (Figures 104 - 105). The solubility of Doxofylline and Salbutamol Sulphate were determined in a variety of solvents as per Indian Pharmacopeial standards. Solubility was carried out in non – polar to polar solvents. Distilled water was selected as a common solvent. The solubility profile of Doxofylline and Salbutamol Sulphate are given in the Table 84 and 85 respectively.

The sample solution of 10 µg/ml of Doxofylline and Salbutamol sulphate were prepared individually and the solutions were scanned between 200 – 400 nm by using water as blank (Figure 106). From the overlaid spectra by observing the spectral characteristics λ max of DOX 274 nm and λ max of SAL 224 nm was selected for simultaneous equation method. The stability of the drug solution was observed at different time intervals.

Doxofylline was stable for 5 hours and Salbutamol Sulphate was stable for 6 hours. From the aliquots of stock solution of DOX and SAL, concentrations (5-25 µg/ml, 5-25 µg/ml) were prepared. The calibration curve was plotted with absorbance versus concentration for the two drugs. The optical characteristics such as correlation coefficient slope, intercept, LOD and LOQ were calculated and regression equation was constructed.

The correlation coefficient was found to be 0.999886 for DOX at 224 nm and 0.9996607 for SAL at 274 nm. At 224 nm the LOD and LOQ were found to be 0.047535 µg/ ml and 0.144046 µg/ ml for DOX. At 274 nm the LOD and LOQ were found to be 0.000927 µg/ ml and 0.002811 µg/ ml for SAL. The correlation coefficient values at all the

selected wavelengths are found to be above 0.999. Hence the selected concentrations are linear and obeyed Beer's law. The calibration graphs for DOX at 224 nm and 274 nm are shown in Figure 108 and 109 respectively.

The calibration graphs for SAL at 224 nm and 274 nm are shown in Figure 110 and 111. The optical characteristic at 224 nm, 274 nm are shown in Tables 86 and 87 respectively. The percentage purity of drugs in the formulation was found to be 99.9685 ± 0.178878 for DOX 100.0 ± 1.118034 for SAL. The developed method was applied for the analysis of synthetic mixture to find out the developed method was correct or not.

The % average of synthetic mixture was found to be 99.884 for DOX and for SAL 100.106 (Table-88). The amount found was good agreement with the expected concentration. Hence it was planned to apply for the analysis of formulation

The precision of the method was confirmed by the repeated analysis of the formulation for six times. The percentage RSD was calculated. The percentage RSD of Doxofylline and were found to be 0.178946 and 1.118034 for SAL respectively. The low % RSD values suggest that the method has good precision. The results are shown in Table 89.

Further, precision of the method was confirmed by Intraday and Inter day analysis. Intraday and Interday analysis of formulation was done on three times on same day and one time on three consecutive days. The percentage RSD for the Intraday and Interday precision of DOX was found to be 0.426135 and for SAL 0.349896 (Table 90). The low % RSD values suggest that the precision of the method was further confirmed. The ruggedness of the method was confirmed by performing the analysis with the different analysts and different instruments.

The % obtained by Different analyst was found to be 101.94 for DOX and 99.00 for SAL (Table 91). The accuracy of the method was confirmed by recovery studies. The percentage recovery was found to be in the range of 99.9776 ± 0.11898 for DOX, 99.840 ± 0.118749 for SAL. The percentage RSD was found to be 0.119007 for DOX and 0.118939 for SAL.

The low percentage RSD indicated that there was no interference due to excipients used in formulation. Hence, the accuracy of the method was confirmed. The data for recovery studies are given in Table 92.

5.5.1.2 AREA UNDER CURVE METHOD

A simple, accurate, rapid precise area under curve method was developed and validated. Distilled water was chosen as a common solvent for the estimation of Doxofylline and Salbutamol Sulphate. The sample solution of 10µg/ml Doxofylline and Salbutamol Sulphate were prepared individually and the solutions were scanned between 200 – 400 nm by using water as a blank. From the overlaid spectra (Figure-106) by observing the spectral characteristics the absorbances are measured at 220 nm - 230 nm for DOX and 270 nm - 280 nm for SAL.

Aliquots of serial dilution were made in the concentration range from 5-25 µg/ml, 5-25 µg/ml. By observing, concentration was proportional to absorbance and it obeys Beer's law. The optical characters were listed in the Table 93 and 94. The correlation co-efficient for DOX was found to be 0.999767 and for SAL 0.9997400 at 220 nm – 230 nm. At 270 nm - 280 nm the LOD and LOQ were 0.00306650 and 0.0092924 for SAL. The calibration graphs for DOX at 220 nm – 230 nm and 270 nm - 280 nm are shown in Figure 112 and 113 and for SAL Figure 114 and 115 respectively.

The developed method was applied for the analysis of synthetic mixture to find out the developed method was correct or not. The % average of synthetic mixture was found and the results obtained were shown in the Table- 95. The amount found was good agreement with the expected concentration. Hence it was planned to apply for the analysis of formulation

The percentage purity of drugs in the formulation was found to be 99.997 ± 0.068148 and 100.2083 ± 1.461306 for DOX and SAL. The precision of the method was confirmed by the repeated analysis of the formulation for six times. The percentage RSD was calculated. The percentage RSD of DOX and SAL were found to be 0.068148 and 0.959025 respectively. The low % RSD values suggest that the method has good precision.

The precision was confirmed by Intraday and Inter day analysis. Intraday and Inter day analysis of formulation was done on three times on same day and one time on three consecutive days. The percentage RSD for the Intraday and Inter day precision was found to be 0.300654 and 0.470304 for DOX and SAL. The low % RSD values suggest that the precision of the method was further confirmed and shown in the Table 97.

The ruggedness study was performed by different instruments and different analyst and the results were listed in the Table. 98. The Accuracy was confirmed by recovery studies. The percentage recovery was found to be in the range of 100. 483 ± 0.446132 for DOX, 99.977 ± 0.331385 for SAL. The % RSD values were found to be 0.443986 and 0.33146 are listed in the Table 99 respectively.

5.5.1.3 DERIVATIVE SPECTROSCOPY METHOD

A simple, accurate, rapid precise method was developed and validated. Distilled water was chosen as a common solvent for the estimation of Doxofylline and Salbutamol Sulphate**.** The sample solution of 10 µg/ml of Doxofylline and Salbutamol Sulphate were prepared individually and the solutions were scanned between 200 – 400 nm by using water as blank. A normal spectrum was derivatised to second order in the UV spectrum of DOX and SAL given in Fig 107, in which 233 nm was selected for the estimation of DOX which is ZCP for SAL and 229 nm was selected for the estimation of SAL which is ZCP for DOX. Experimental conditions describes, calibration curve, assay of tablets, recovery studies, precision studies, LODs & LOQs.

A critical evaluation of proposed method was performed statistical analysis of data where slope intercept correlation coefficient was studied. Beer's law obeys in the concentration range of 5 - 25 µg/ml, 5-25 µg/ml for each drug and correlation coefficient was 0.9998851 for DOX and 0.999794 for SAL (Table.100). The calibration graphs for DOX at 229 and for SAL at 233nm were shown in the Figure 116 and 117. The analysis of synthetic mixture results was shown in the Table-101.

The proposed method was also evaluated by assay of commercially available tablets containing DOX & SAL (n=6). The precision of the method was confirmed by the repeated analysis of the formulation for six times. The percentage RSD was calculated. The percentage purity of drugs in the formulation was found to be 99.9858 ± 0.169364 for DOX and 99.70833 ± 0.79713 for SAL are listed in Table 102.

The precision was confirmed by Intraday and Inter day analysis. Intraday and Inter day analysis of formulation was done on three times on same day and one time on three

consecutive days. The percentage RSD for the Intraday and Inter day precision was found to be 0.887693 and 0.12545 for DOX and SAL was 0.179188 and 0.3261 (Table-103) respectively.

The Ruggedness study was performed and the results were shown in the Table 104. The % recovery assay was found to be 100.0733 for DOX and 99.7943 for SAL. The % RSD value was found to be 0.127317 for DOX and 0.1917 for SAL (Table 105)

SUMMARY

&

CONCLUSION

6. SUMMARY AND CONCLUSION

Simple, precise and accurate methods were developed for following combination in bulk and in pharmaceutical dosage form.

1. TOLPERISONE HYDROCHLORIDE AND PARACETAMOL
2. SITAGLIPTIN PHOSPHATE AND SIMVASTATIN
3. THIOCOLCHICOSIDE AND KETOPROFEN
4. DESLORATADINE AND AMBROXOL HYDROCHLORIDE
5. DOXOFYLLINE AND SALBUTAMOL SULPHATE

6.1 TOLPERISONE HYDROCHLORIDE AND PARACETAMOL

From the solubility data, Distilled water was selected as solvent. From the overlaid spectra, the wavelengths selected were 261 nm and 243 nm for simultaneous equation method**.** For the Absorption ratio method, one is the λ max of one of the component Paracetamol, λ_2 243 nm and the other being an equal absorptivity of two component λ_1 Tolperisone Hydrochloride 254 nm an iso-absorptive point. For the Area under curve method, the wavelength selected were 253 nm – 269 nm for TPE and 274 nm – 284 nm for PCL. For Derivative spectroscopic methods, the wavelength 261 nm was selected for the estimation of PCL, which is the zero crossing point for TPE and 243 nm was selected for the estimation of TPE which is zero crossing point for PCL**.**

In RP-HPLC method, the conditions were optimized to obtain an adequate separation of eluted title compounds. The optimization was done by changing the composition of mobile phase. The mobile phase consists of Methanol: Acetonitrile with 0.1 ml of 0.1% triethylamine with the ratio of 60: 40% v/v. The retention time for

Tolperisone Hydrochloride and Paracetamol was found to be 2.915 and 4.637 minutes respectively and with a resolution of 9.087 which is better resolution.

In HPTLC method, after various trials Chloroform: acetic acid: water (6:2:2v/v/v) was selected. UV spectra of both the drugs were recorded and overlaid. The percentage RSD values were found to be 0.24248 and 0.472852 for Tolperisone Hydrochloride and Paracetamol, respectively. The low percentage RSD value indicates that there was no interference due to the excipients used in formulation during the analysis.

6.2 SIMVASTATIN AND SITAGLIPTIN PHOSPHATE

For Derivative method, the common solvents were found to be as methanol for the analysis of Simvastatin and Sitagliptin phosphate for the proposed method. The zero order spectra obtained and it was derivatised to first order spectrum. The values of amplitudes were measured for Sitagliptin Phosphate at 277 nm and 238 nm measured for Simvastatin respectively.

In RP-HPLC method, after calculating all system suitability parameters the mobile phase Methanol: Acetonitrile with 0.1 ml of 0.1% Triethylamine in the ratio of 60: 40 % v/v at flow rate of 1.0 ml/ min was selected. The retention time for SITA and SIM was found to be 4.03 and 6.8 minutes respectively.

In HPTLC method, after various trials Toluene: methanol: acetic acid: (5:4:1 v/v/v) was selected. From the overlain spectra, at 255 nm both the drugs showed marked absorbance. The low percentage RSD value indicates that there was no interference due to the excipients used in formulation during the analysis.

6.3 THIOCOLCHICOSIDE AND KETOPROFEN

Methanol and water was chosen as a common solvent for the estimation of Thiocolchicoside and Ketoprofen. The values of amplitudes were measured for THI at 233.5 nm and 259 nm measured for KET.

For HPLC method, the mobile phase consists of Acetonitrile: Water (60:40 v/v) provided optimum polarity for proper migration, separation and resolution of Thiocolchicoside and Ketoprofen. The retention time for THI and KET was found to be 3.743 and 7.903 minutes respectively.

6.4 DESLORATADINE AND AMBROXOL HYDROCHLORIDE

For the overlaid spectrum, in the Absorption ratio method, the absorbance's are measured at two wavelengths one being the λ max of one of the component Ambroxol, λ_2 244 nm and the other being a equal absorptive of two component λ_1 Desloratadine 288 nm an isobestic point**.** The zero order spectra obtained and it was derivatised to first order spectrum. The values of amplitudes were measured for Ambroxol at 320 nm and 277 nm for Desloratadine respectively**.**

6.5 DOXOFYLLINE AND SALBUTAMOL SULPHATE

From the overlaid spectra the wavelengths 274 nm and 224 nm were selected for the formation of Simultaneous equation method. For the Area under curve method, the wavelength selected were 220-230 nm for DOX and 270 nm – 280 nm for SAL. For Derivative Spectroscopic method, the zero order spectra was derivatised to second order spectra in that 233 and 229 nm was selected for the estimation of SAL and DOX.

Three instrumental analytical methods were successfully developed for the simultaneous estimation of in bulk and in pharmaceutical dosage form.

The above described methods were found to be accurate, precise and rapid for the simultaneous estimation of those combination drugs. The results drawn were confirmed by low percentage RSD values. The spectrophotometric method was found to be economical when compared to the HPLC and HPTLC method. But HPLC and HPTLC is more sensitive than UV spectrophotometric method. The low percentage RSD value in the recovery studies suggests that the excipients present in the dosage forms do not interfere in the analysis of formulation and hence all the methods are accurate. The linearity range, LOD, LOQ were less in HPLC and HPTLC method while compared to UV spectroscopic method. Hence it was suggested that the developed methods can be applied successfully for the routine quality control analysis for the simultaneous estimation of drugs in bulk and in Pharmaceutical dosage form and the obtained results can be presented elsewhere.

IMPACT OF THE STUDY

7. IMPACT OF THE STUDY

The present work involves Simultaneous Estimation of Newer Analytical method for the new combination of drugs. The combination of drug most commonly refers to a fixed dose combination (FDC) which is formulation including two or more active pharmaceutical ingredients. By using the combination of drugs to analyse, it was more advantageous than the use of individual drug. The drugs were analysed to determine the purity, sensitivity and efficacy.

The current trend followed by the industries is developing a methodology which can save sophisticated instruments and chemist's valuable time by which the product analysis can be done very fast, thereby saving the time phase. UV, HPLC and HPTLC methods are involved in the drug estimation, which is fast as well as novel.

So, this research work was mainly focussed towards the Analytical method development and validation of the combined dosage forms. Three Instruments have been used for the method development. The developed methods are not official in any Pharmacopoeia.

Keeping all these points in mind, the current method has been developed and it is very fast and encouraging. The developed method was validated with a holistic approach according to ICH guidelines and details of findings are expressed.

The methods developed are economical as it requires small amount of solvents with minimum sample to clean up. Its main advantages are that large number of samples can be simultaneously analysed, simplicity and less time-consuming procedure are described. This is the reason why people are more attracted towards analytical method development.

In the methods described above, the samples utilized are in microgram, microlitre and nanograms. The instruments used are highly sensitive to show the interferences of some other excipients and other drug substance.

The novel UV, HPLC and HPTLC methods developed is sensitive, unique, precise, user friendly, rapid, and reproducible for simultaneous estimation of drugs in bulk mix and Pharmaceutical dosages forms. The method was validated as per the ICH Guidelines.

The validated methods produce results within known uncertainties that are helpful to continuing drug development and provide emerging knowledge supporting the product. The time and effort that is devoted into developing scientifically sound and robust analytical methods should be aligned with the drug development stage. The resources that are constantly used during the method development and validation must be balanced with regulatory requirements and the probability for product commercialization.

It is concluded that this method can be used by the industries and academic institutions. The results from these processes are applied by quality control laboratories to ensure the identity, purity, potency, and performance of drug products

With continuation of the research work, in future may be planned to develop method using Biological samples, and Stability indicating methods with various parameters.

FIGURES

Fig - 1 IR SPECTRA OF TOLPERISONE HYDROCHLORIDE

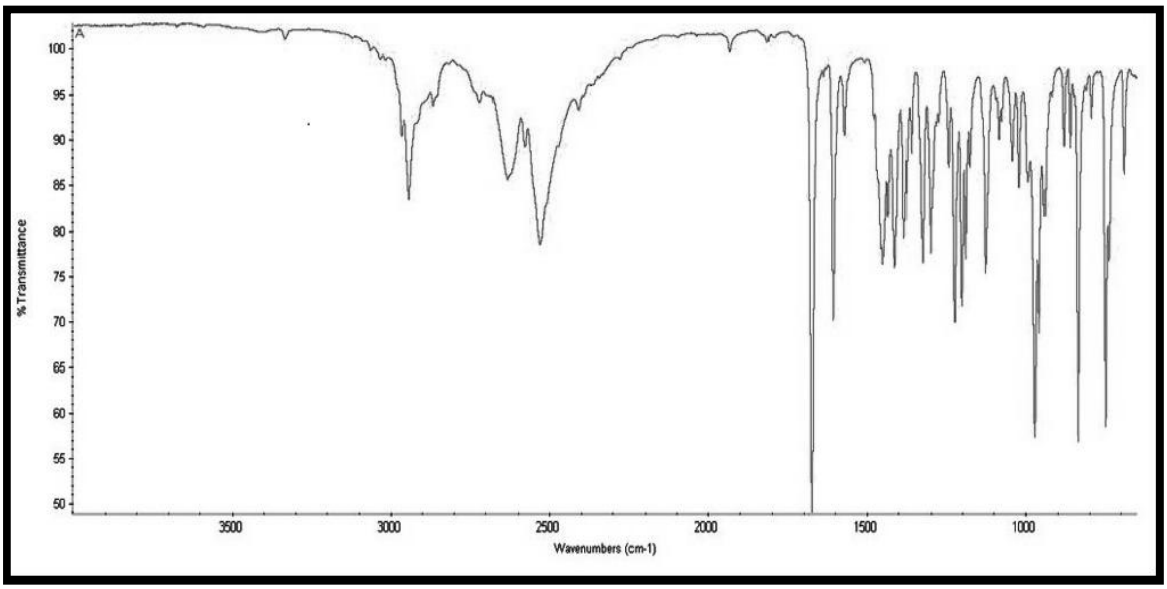


Fig - 2 IR SPECTRA OF PARACETAMOL

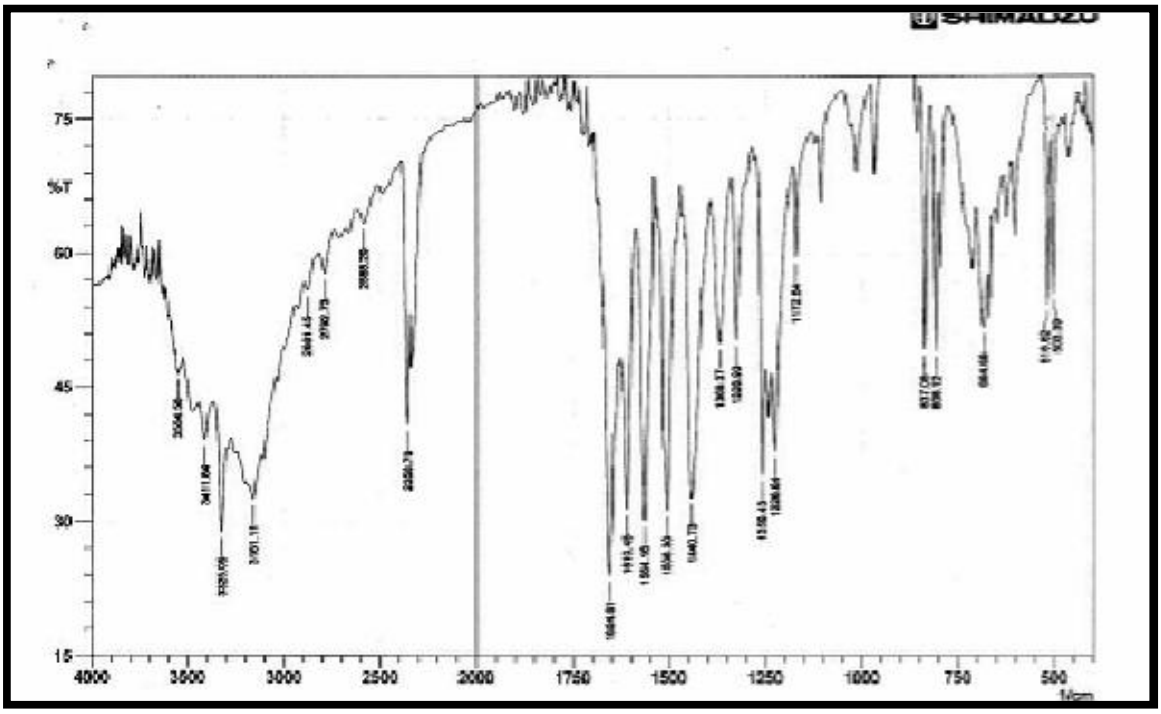


Fig - 3 OVERLAIN SPECTRA OF TOLPERISONE HYDROCHLORIDE AND PARACETAMOL

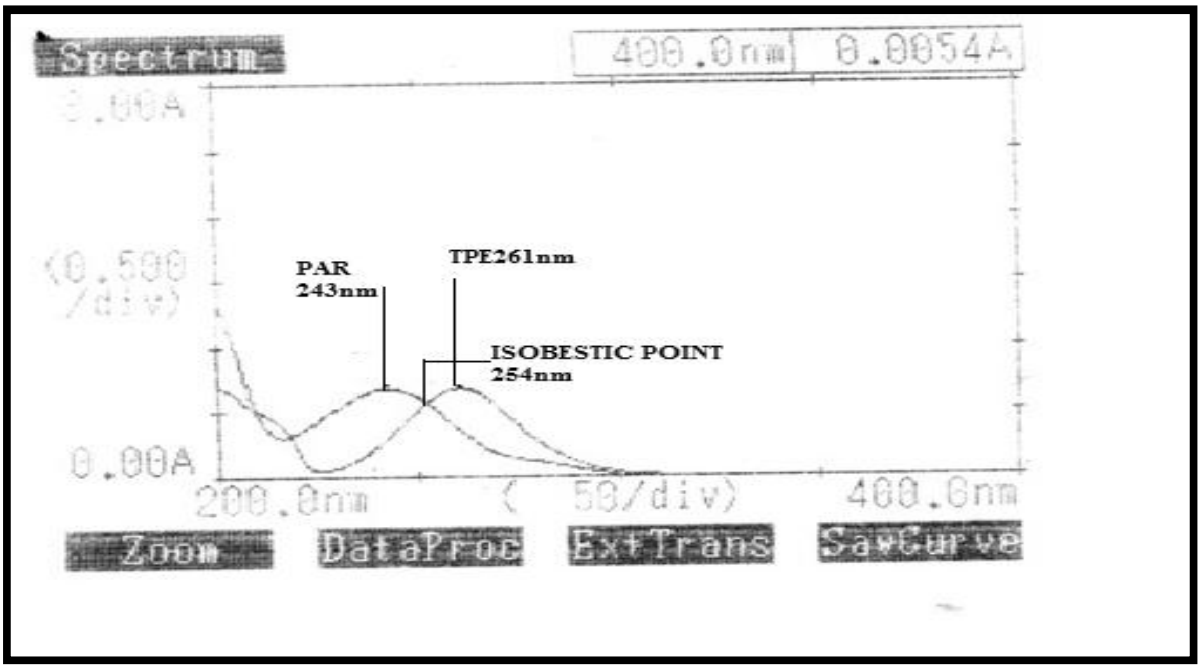


Fig - 4 FIRST ORDER DERIVATIVE SPECTRA OF TOLPERISONE HYDROCHLORIDE AND PARACETAMOL

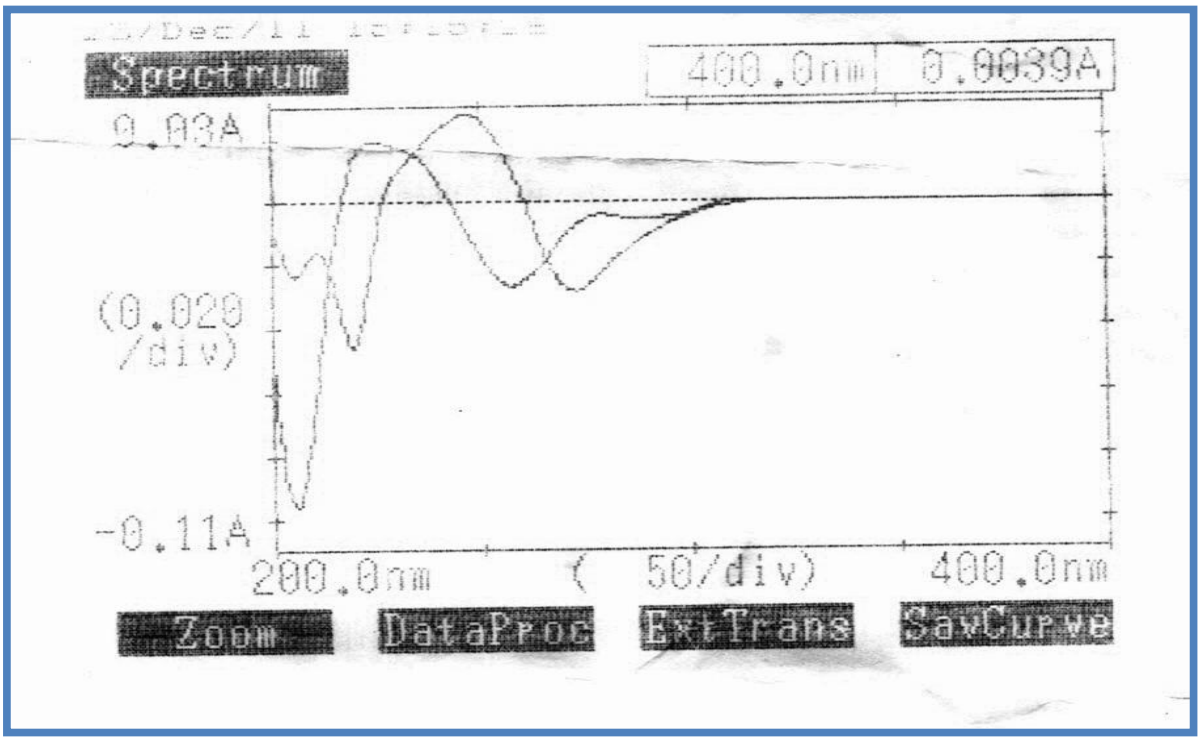


FIG – 5 CALIBRATION CURVE OF TOLPERISONE HYDROCHLORIDE AT 261 nm (SIMULTANEOUS EQUATION METHOD)

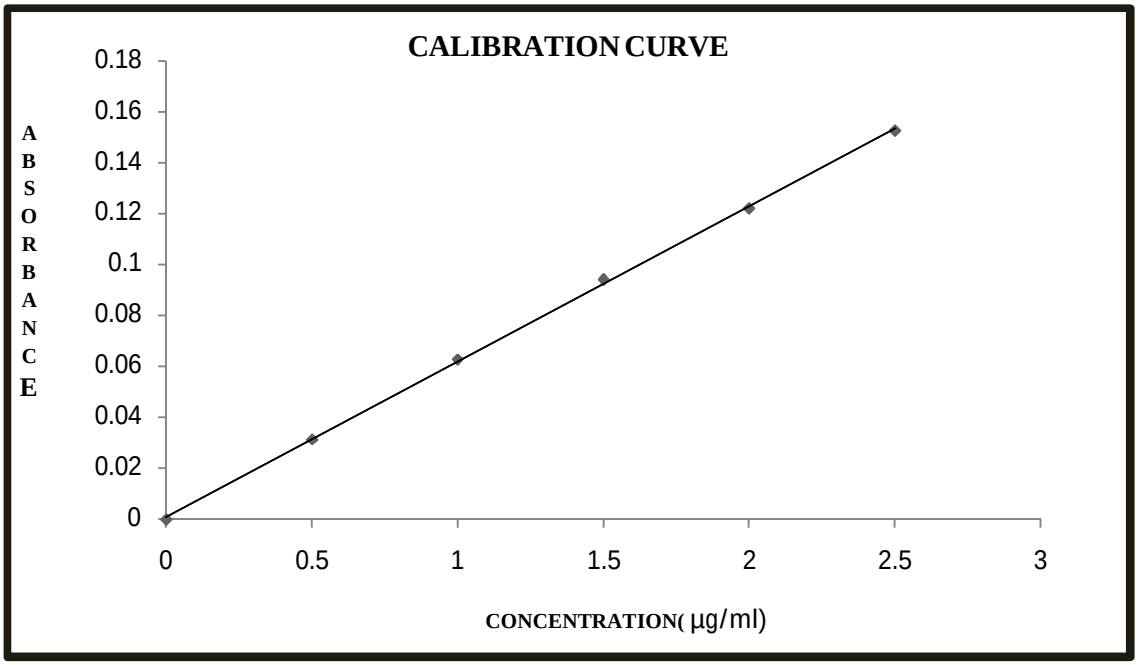


FIG - 6 CALIBRATION CURVE OF TOLPERISONE HYDROCHLORIDE AT 243 nm (SIMULTANEOUS EQUATION METHOD)

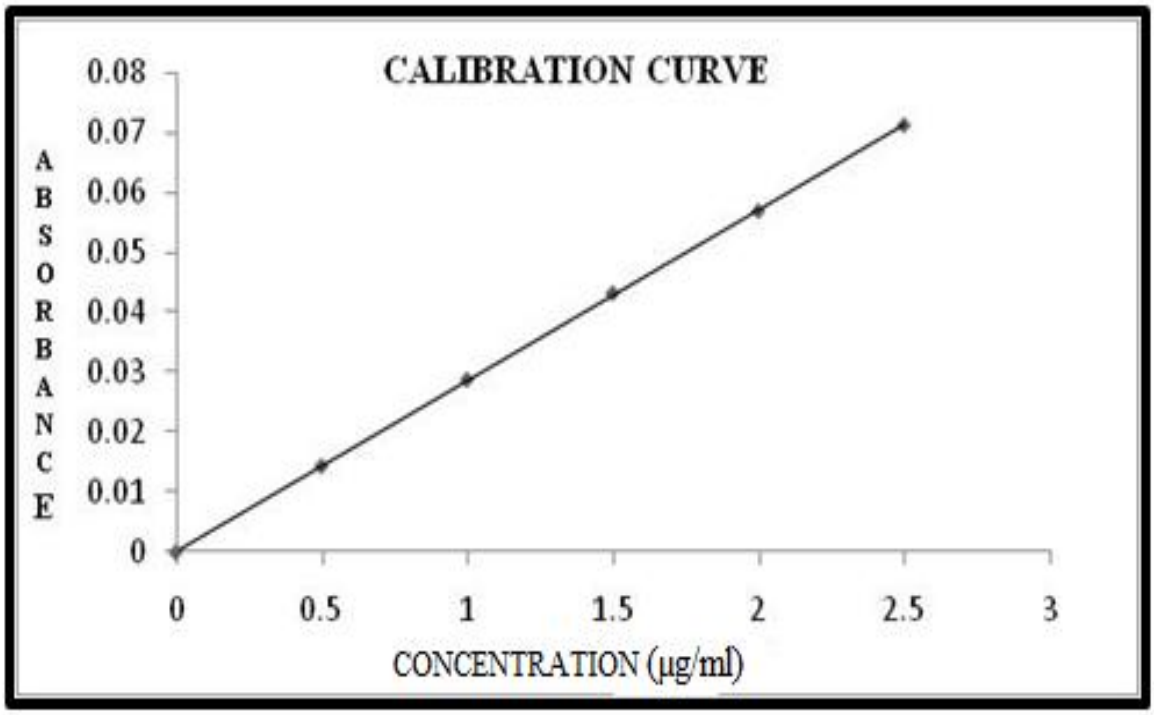


FIG – 7 CALIBRATION CURVE OF PARACETAMOL AT 261 nm (SIMULTANEOUS EQUATION METHOD)

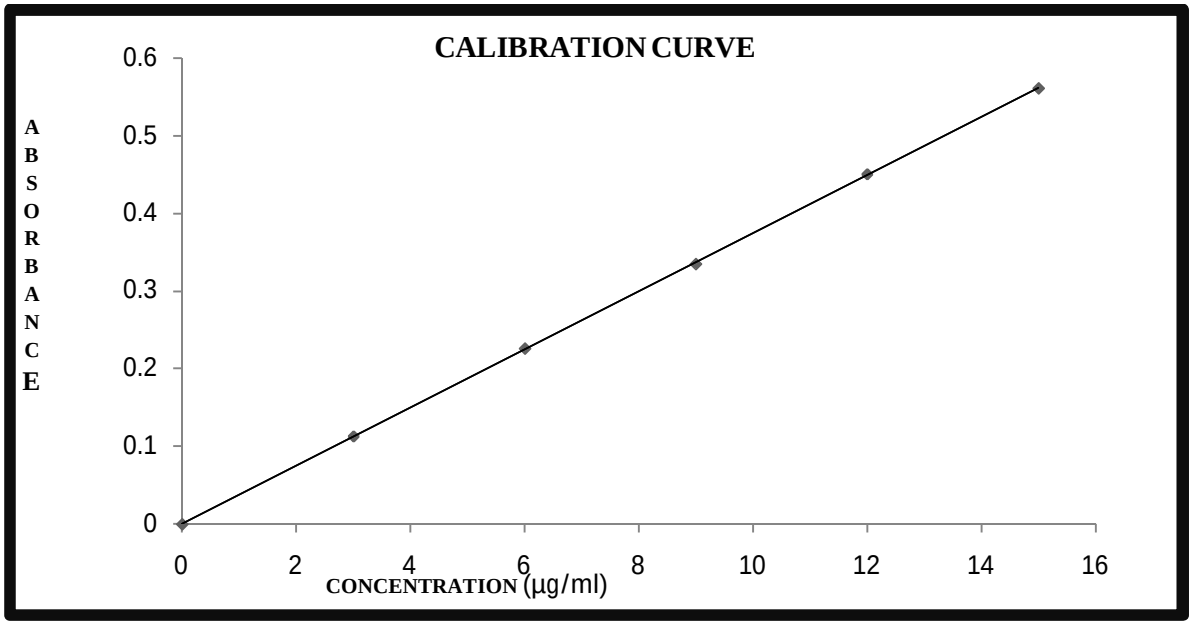


FIG - 8 CALIBRATION CURVE OF PARACETAMOL AT 243 nm (SIMULTANEOUS EQUATION METHOD

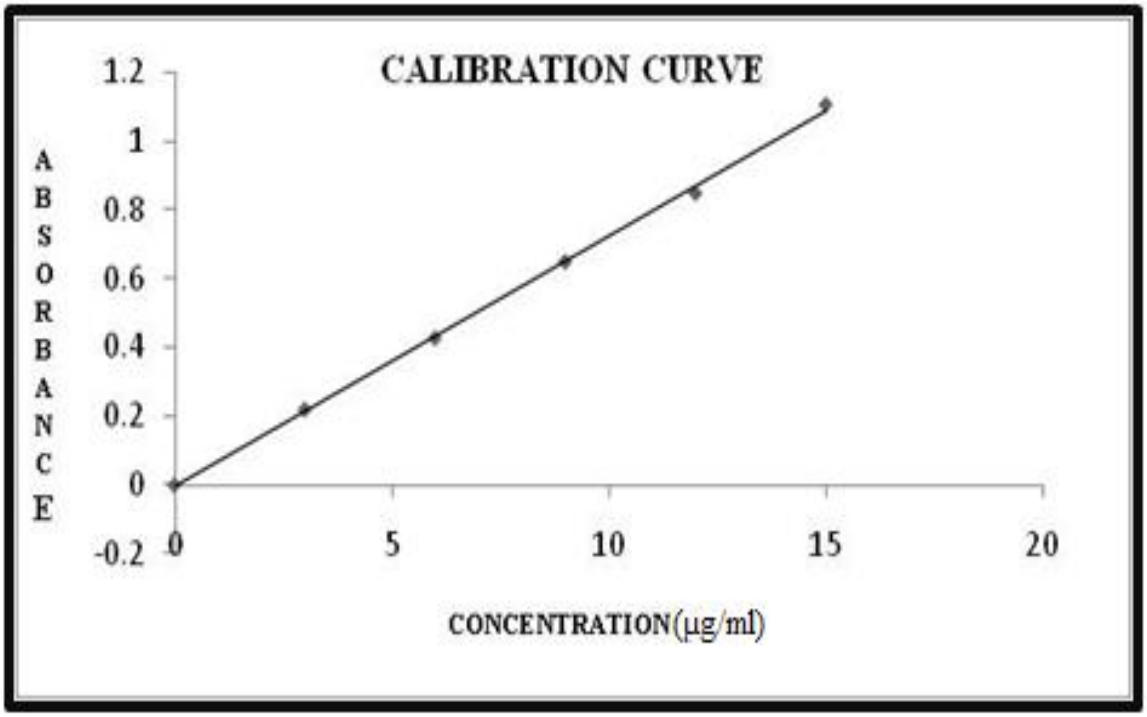


FIG - 9 CALIBRATION CURVE OF PARACETAMOL AT 254 nm (ABSORBANCE RATIO METHOD)

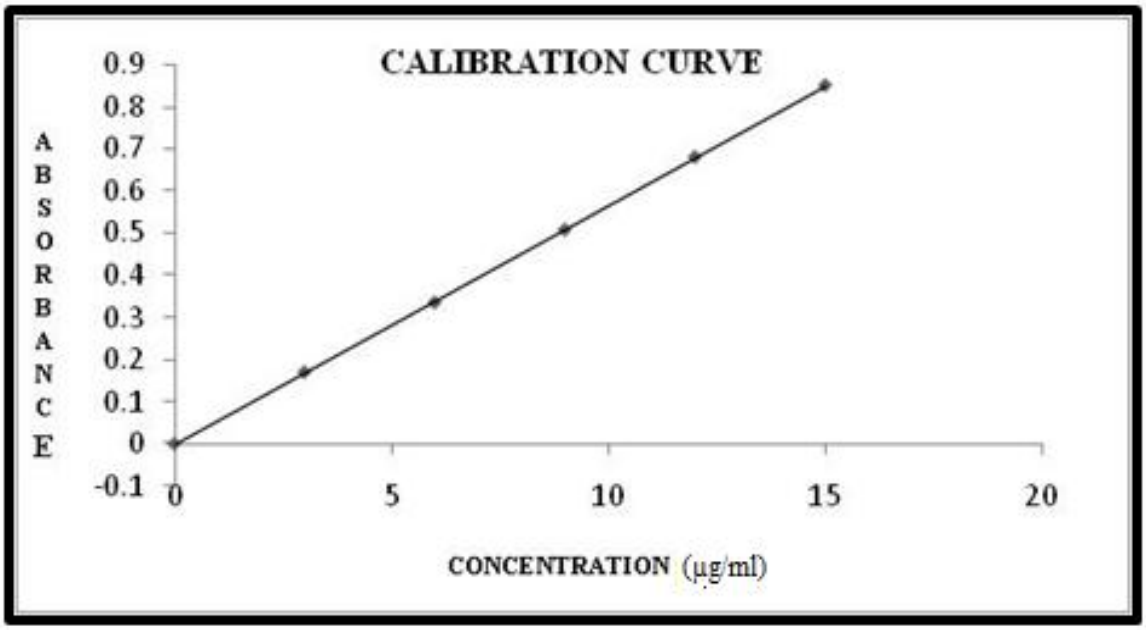


FIG - 10 CALIBRATION CURVE OF TOLPERISONE HYDROCHLORIDE AT 254 nm (ABSORBANCE RATIO METHOD)

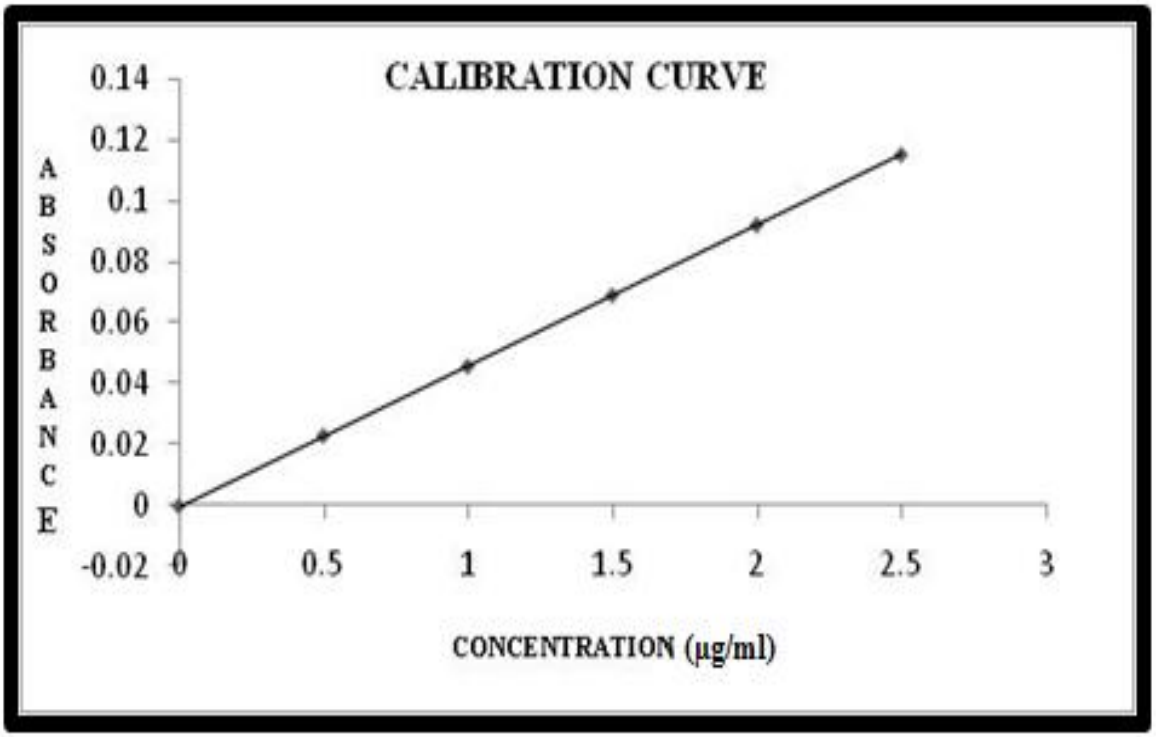


FIG - 11 CALIBRATION CURVE OF PARACETAMOL (DERIVATIVE METHOD)

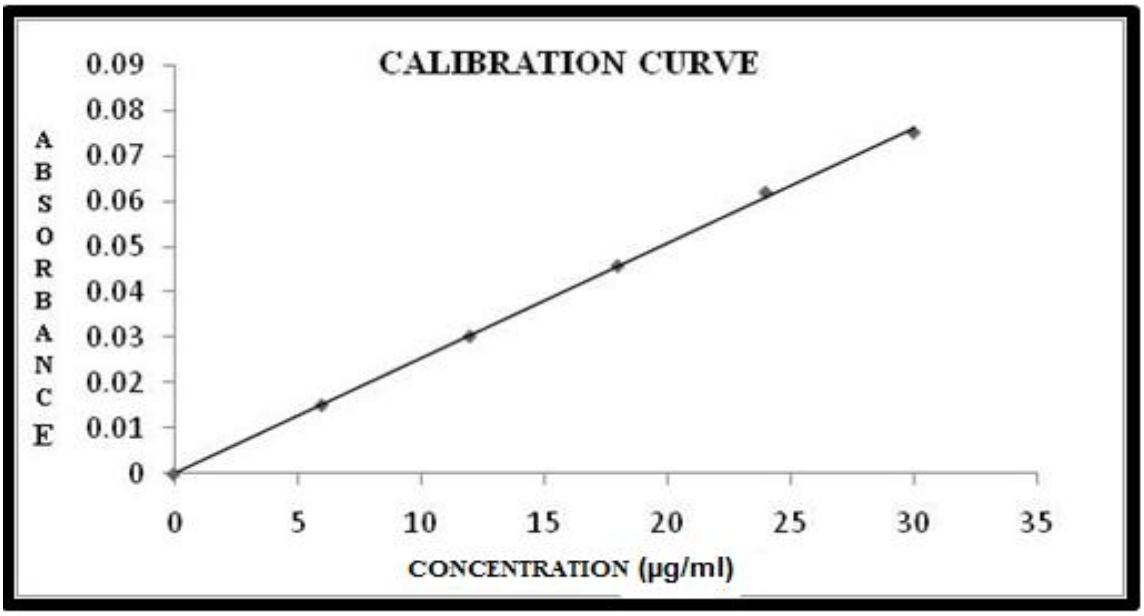


FIG – 12 CALIBRATION CURVE OF TOLPERISONE HYDROCHLORIDE (DERIVATIVE METHOD)

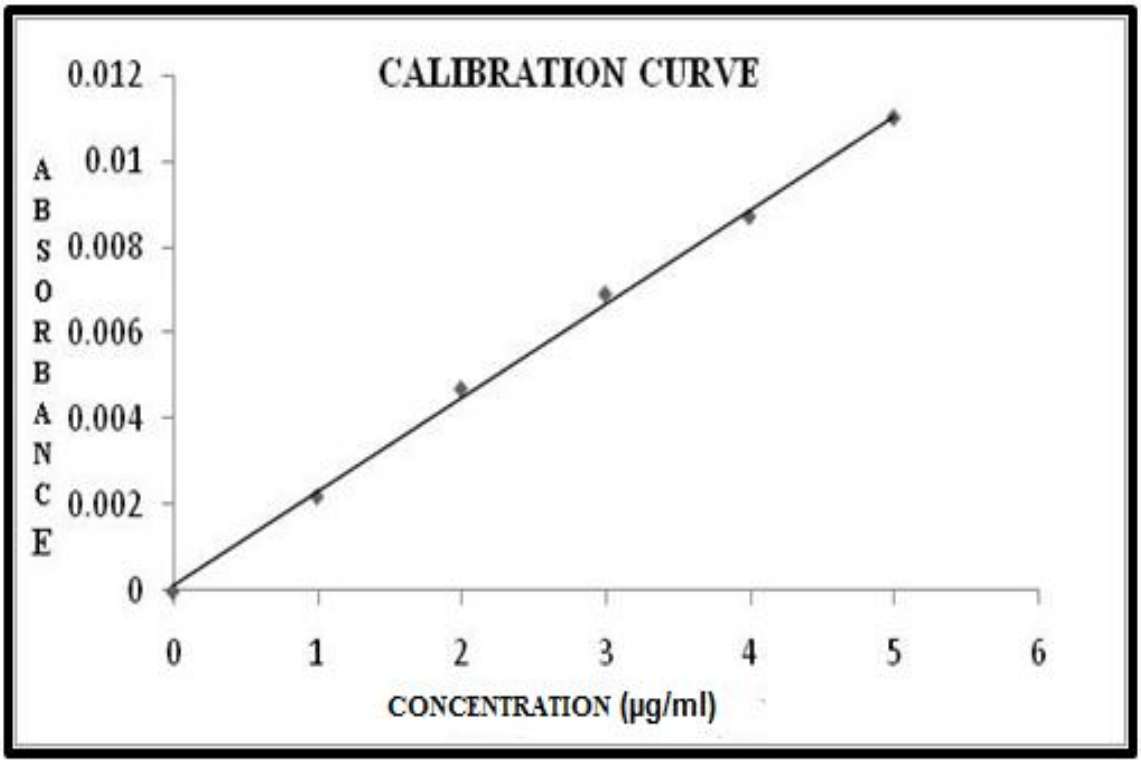


FIG - 13 CALIBRATION CURVE OF TOLPERISONE HYDROCHLORIDE AT 253 nm - 269 nm (AREA UNDER CURVE METHOD)

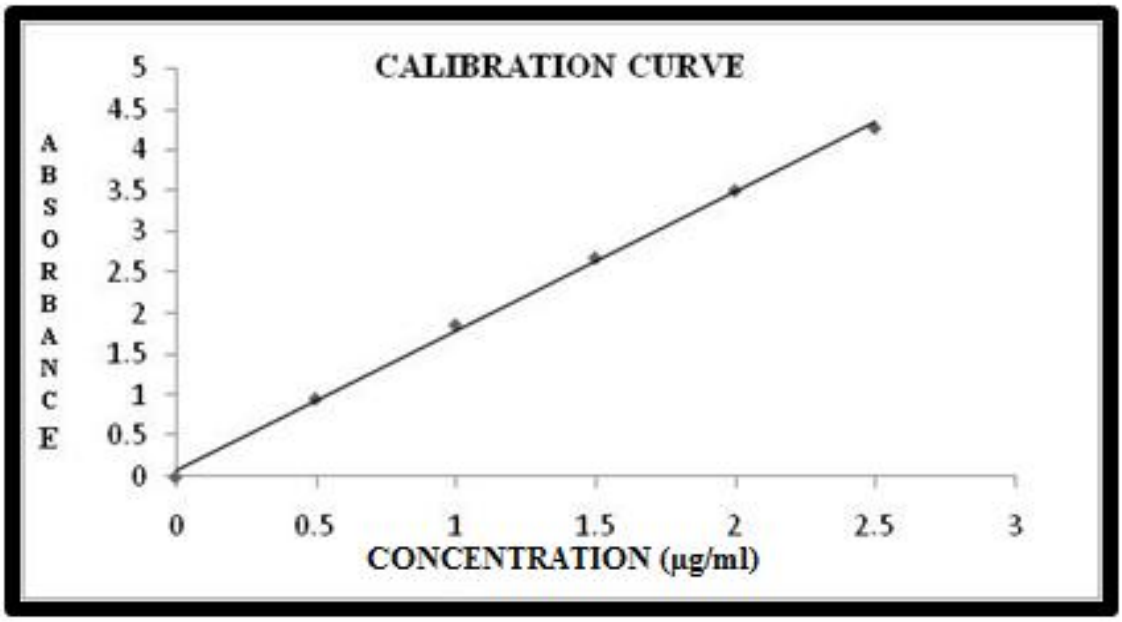


FIG -14 CALIBRATION CURVE OF TOLPERISONE HYDROCHLORIDE AT 274 nm - 284nm (AREA UNDER CURVE METHOD)

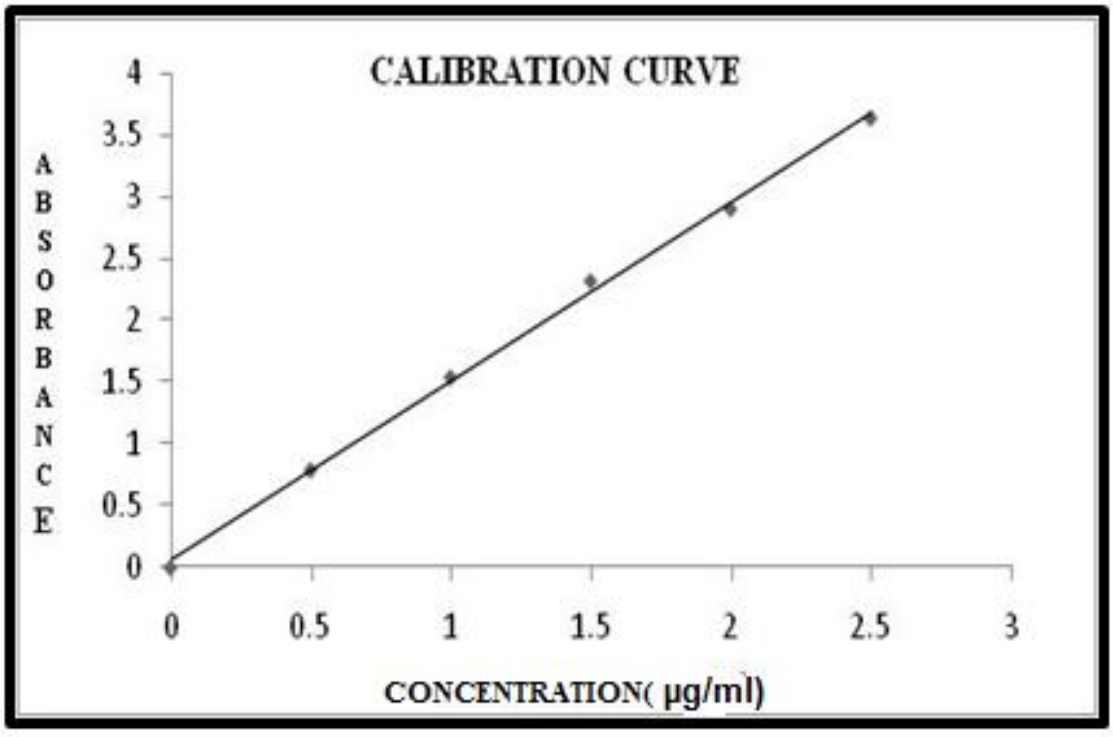


FIG - 15 CALIBRATION CURVE OF PARACETAMOL AT 253 – 269 nm (AREA UNDER CURVE METHOD)

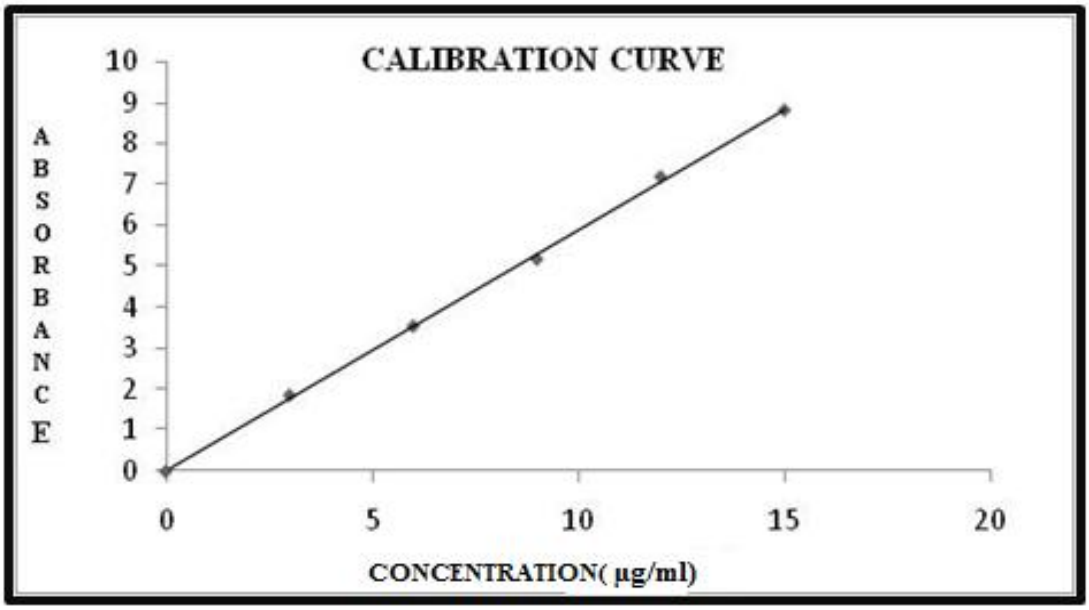


FIG -16 CALIBRATION CURVE OF PARACETAMOL AT 274 nm - 284nm (AREA UNDER CURVE METHOD)

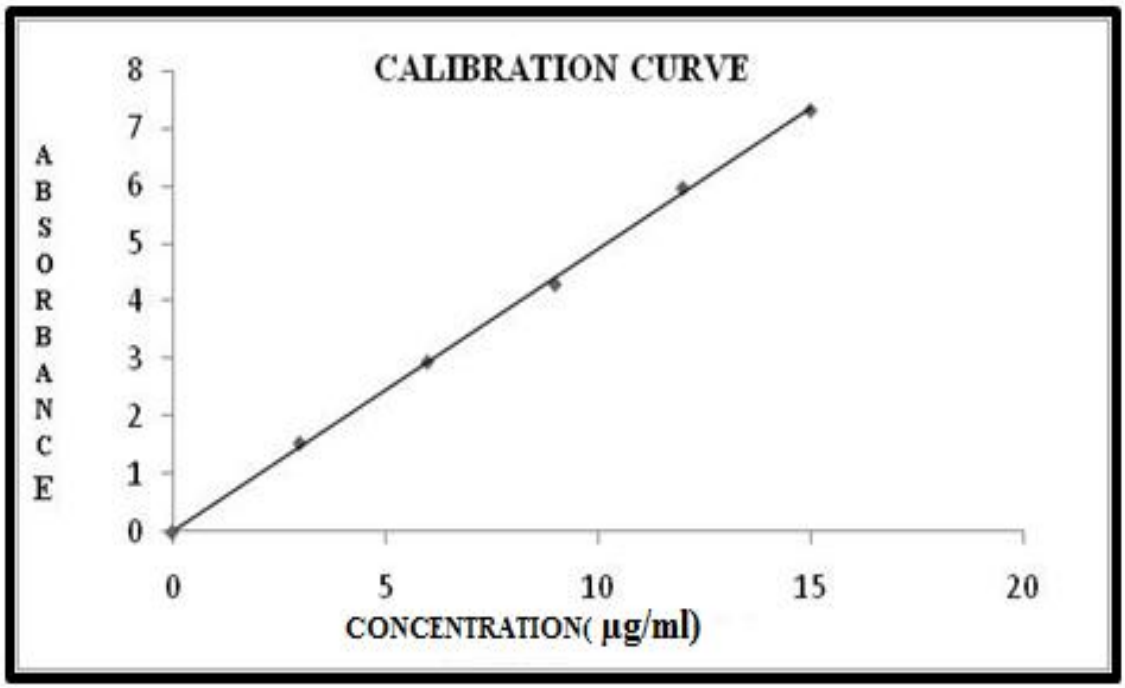
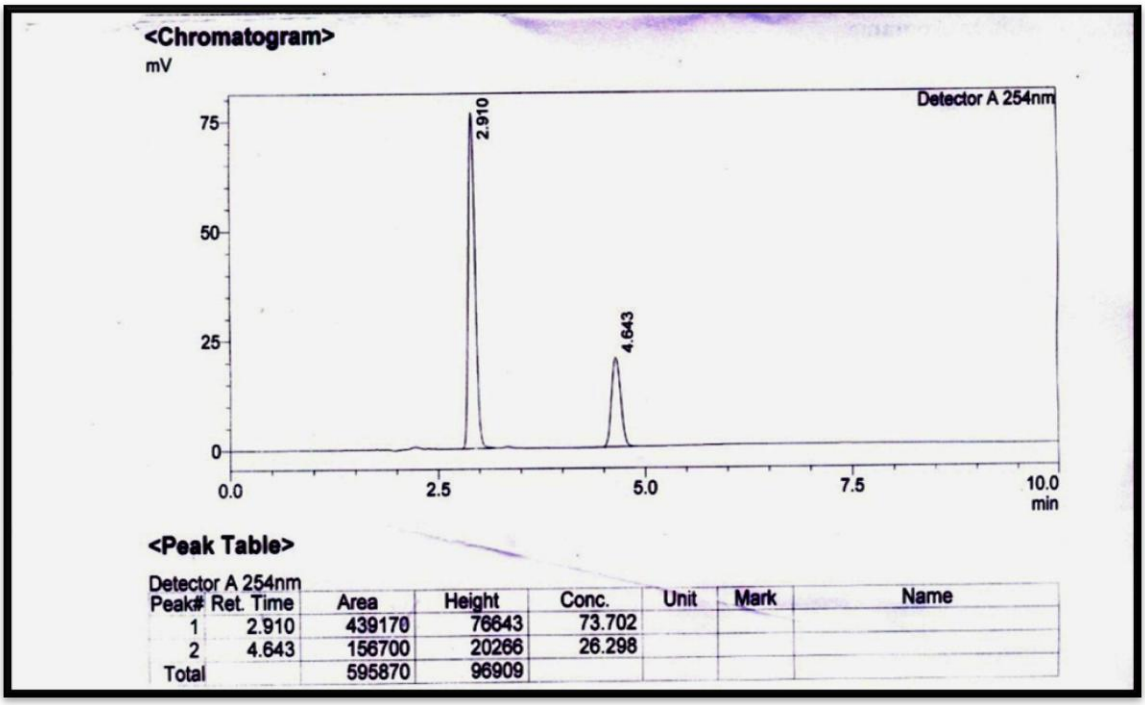


FIG - 17 RP-HPLC LINEARITY CHROMATOGRAM OF TOLPERISONE HYDROCHLORIDE AND PARACETAMOL (2 µg/ml + 4 µg/ml)

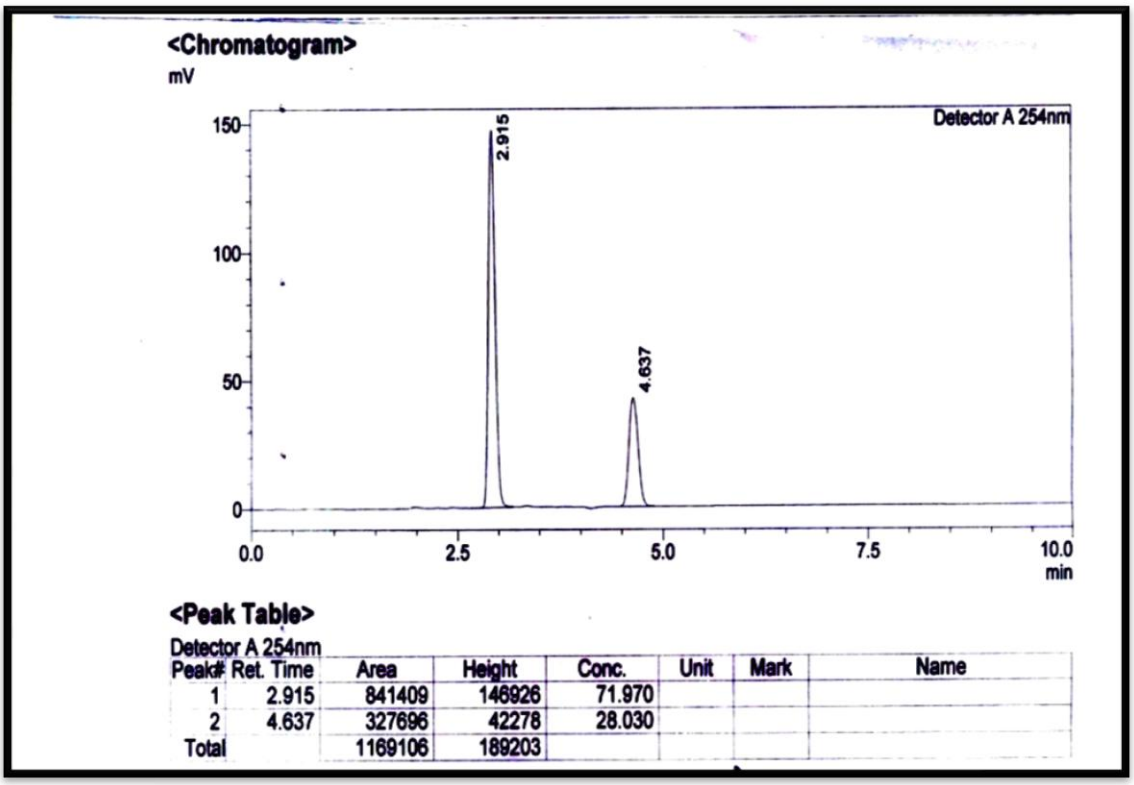


<Peak Table>

Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	2.910	439170	76643	73.702			
2	4.643	156700	20266	26.298			
Total		595870	96909				

FIG - 18 RP- HPLC LINEARITY CHROMATOGRAM OF TOLPERISONE HYDROCHLORIDE AND PARACETAMOL (4 µg/ml +8 µg/ml)

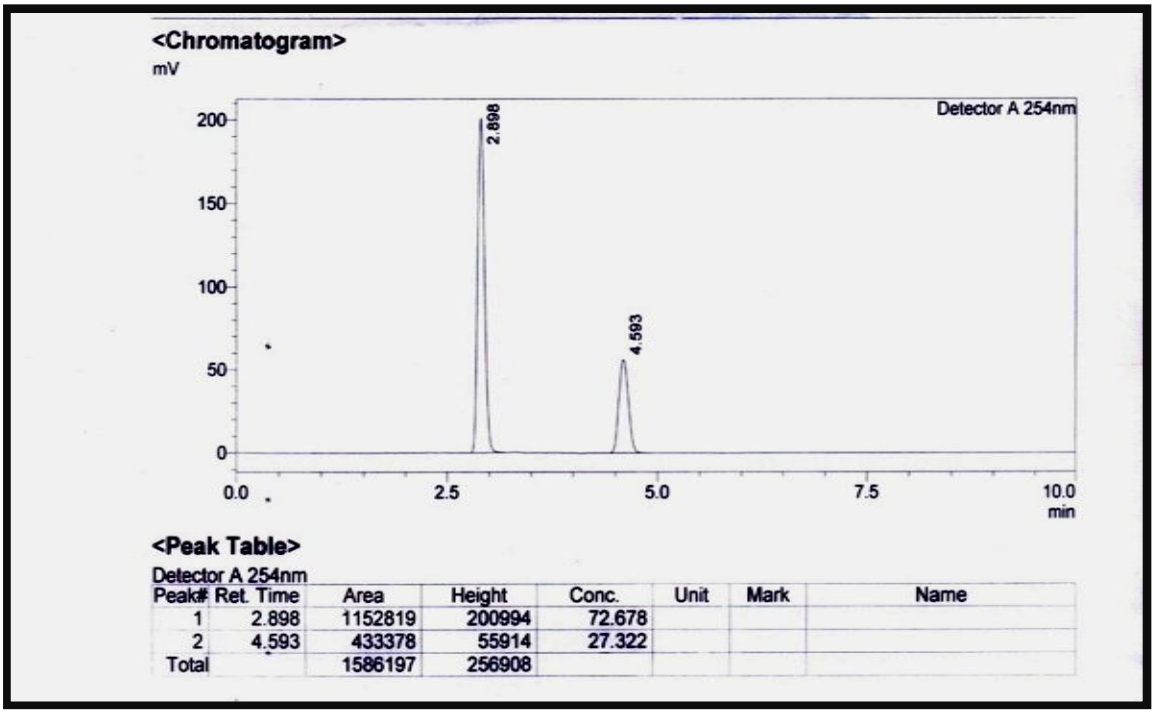


<Peak Table>

Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	2.915	841409	146926	71.970			
2	4.637	327696	42278	28.030			
Total		1169106	189203				

FIG - 19 RP-HPLC LINEARITY CHROMATOGRAM OF TOLPERISONE HYDROCHLORIDE AND PARACETAMOL (6µg/ml +12 µg/ml)

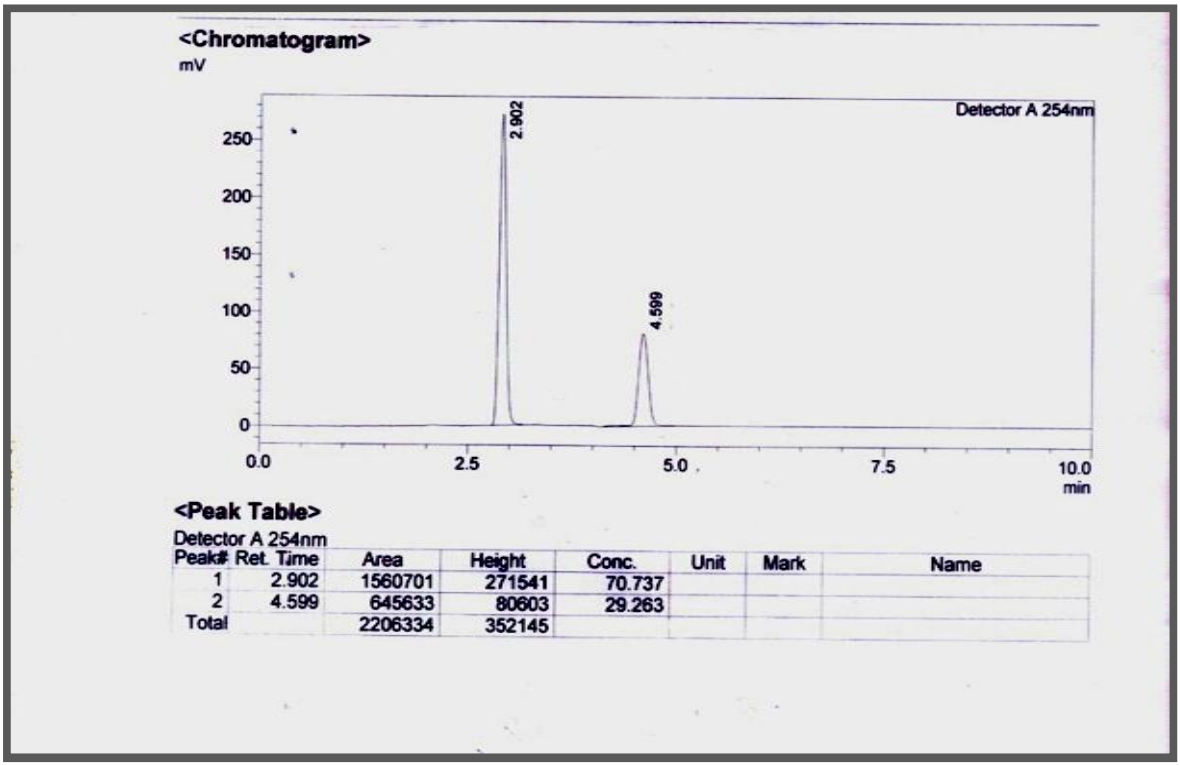


<Peak Table>

Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	2.898	1152819	200994	72.678			
2	4.593	433378	55914	27.322			
Total		1586197	256908				

FIG – 20 RP-HPLC LINEARITY CHROMATOGRAM OF TOLPERISONE HYDROCHLORIDE AND PARACETAMOL (8µg/ml +16 µg/ml)

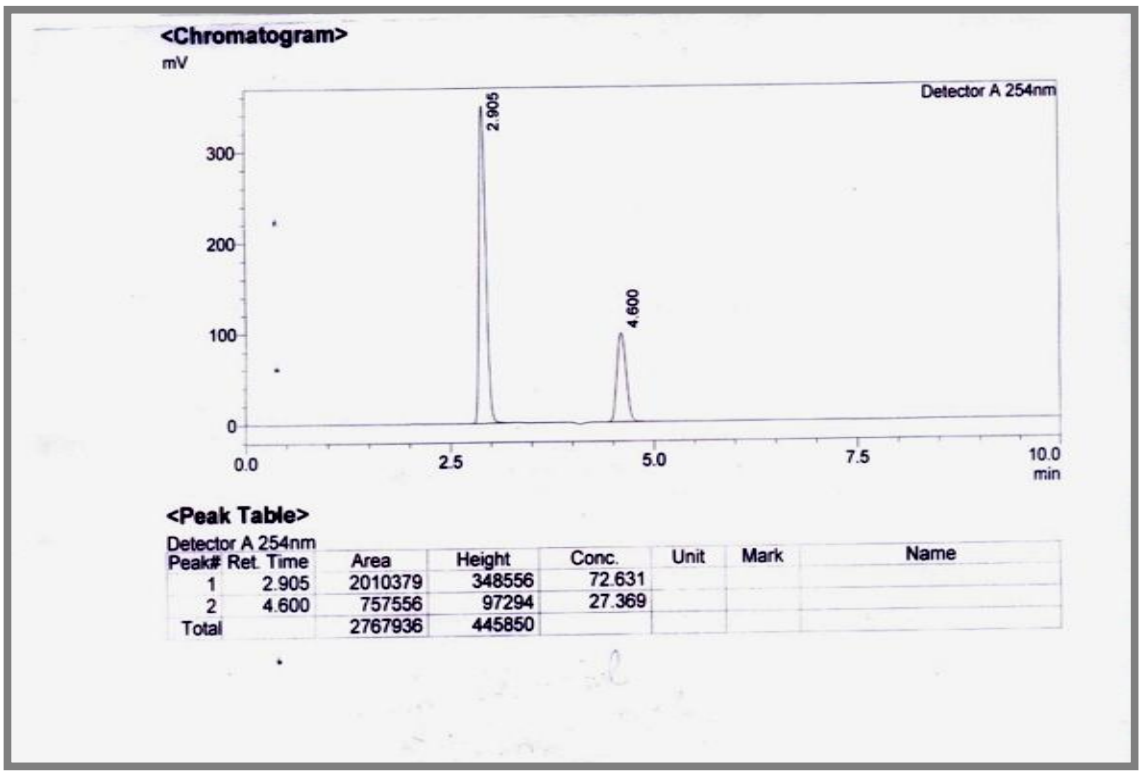


<Peak Table>

Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	2.902	1560701	271541	70.737			
2	4.599	645633	80603	29.263			
Total		2206334	352145				

FIG – 21 RP-HPLC LINEARITY CHROMATOGRAM OF TOLPERISONE HYDROCHLORIDE AND PARACETAMOL (10µg/ml +20 µg/ml)



<Peak Table>

Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	2.905	2010379	348556	72.631			
2	4.600	757556	97294	27.369			
Total		2767936	445850				

FIG - 22 CALIBRATION CURVE OF TOLPERISONE HYDROCHLORIDE BY RP-HPLC METHOD

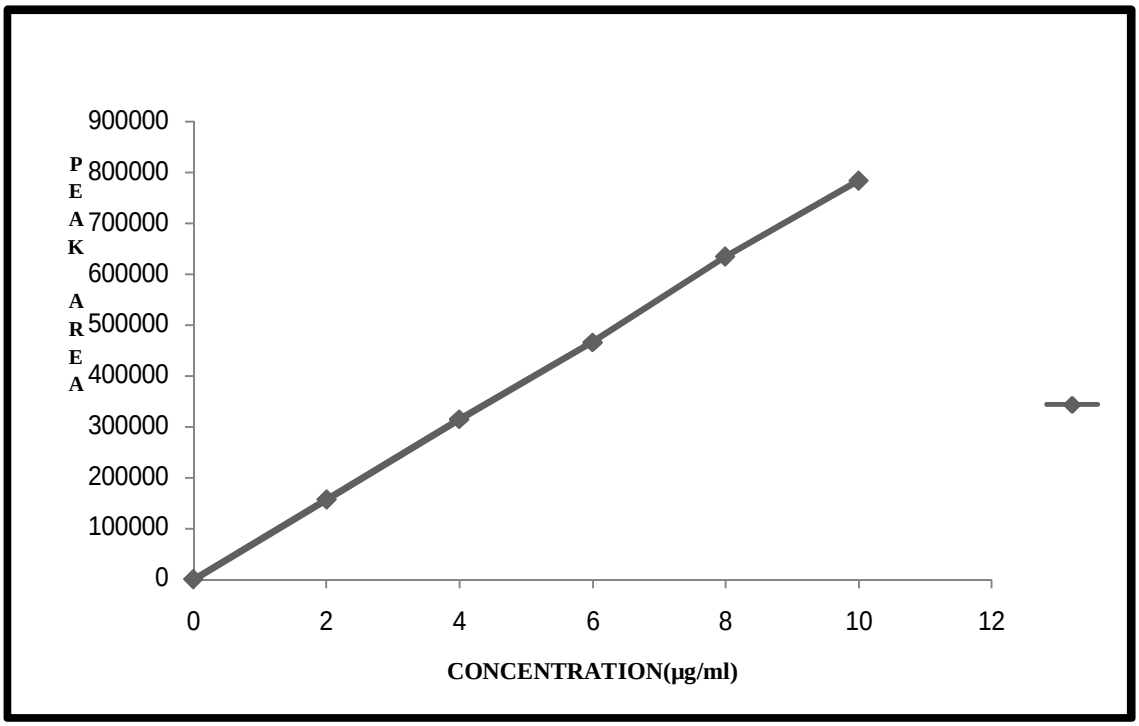


FIG - 23 CALIBRATION CURVE OF PARACETAMOL BY RP-HPLC METHOD

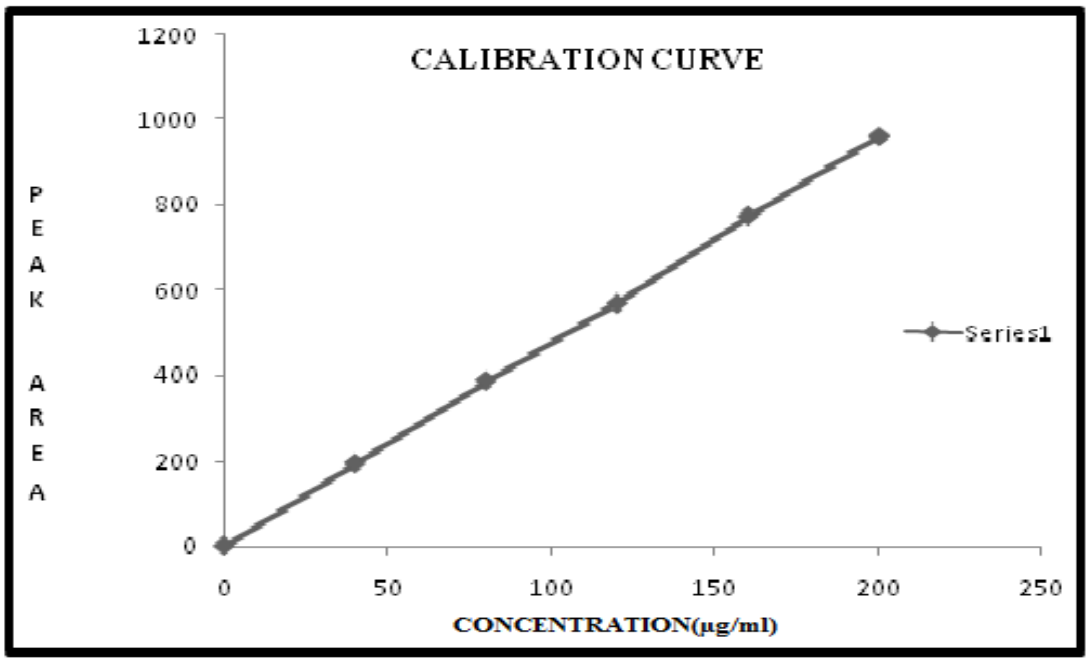
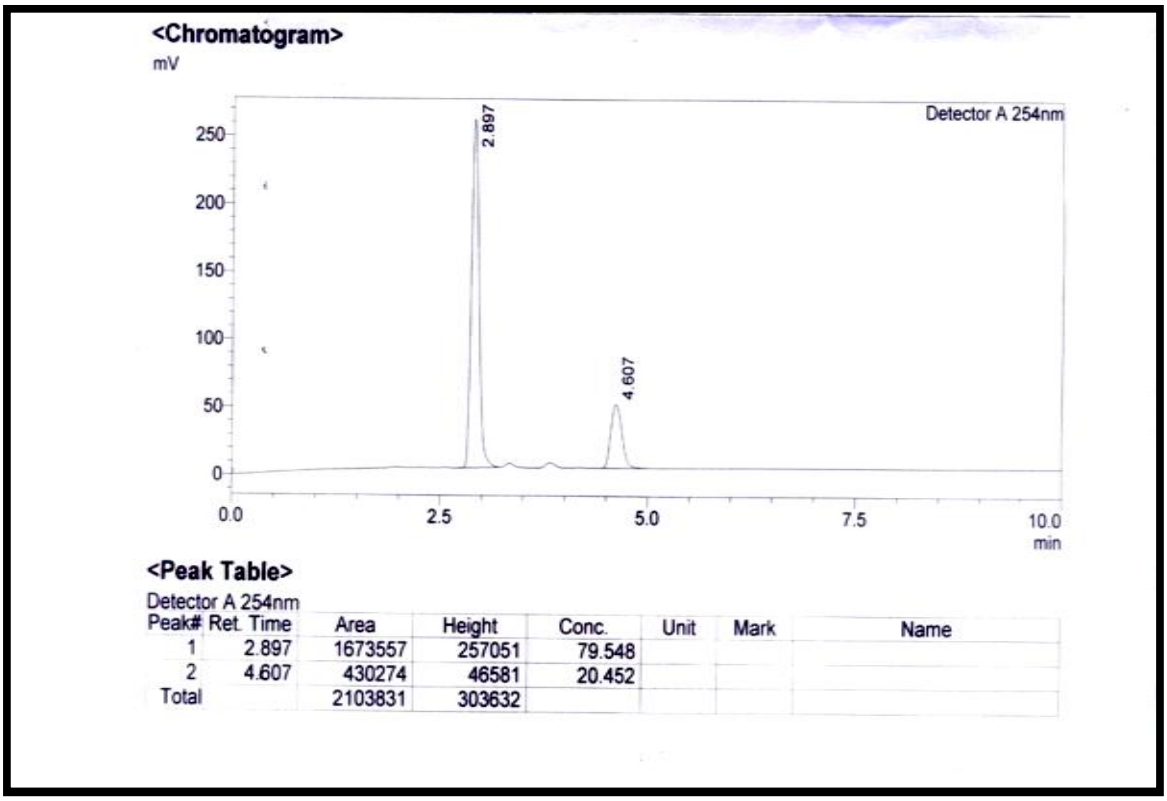


FIG - 24 ANALYSIS OF FORMULATION - I

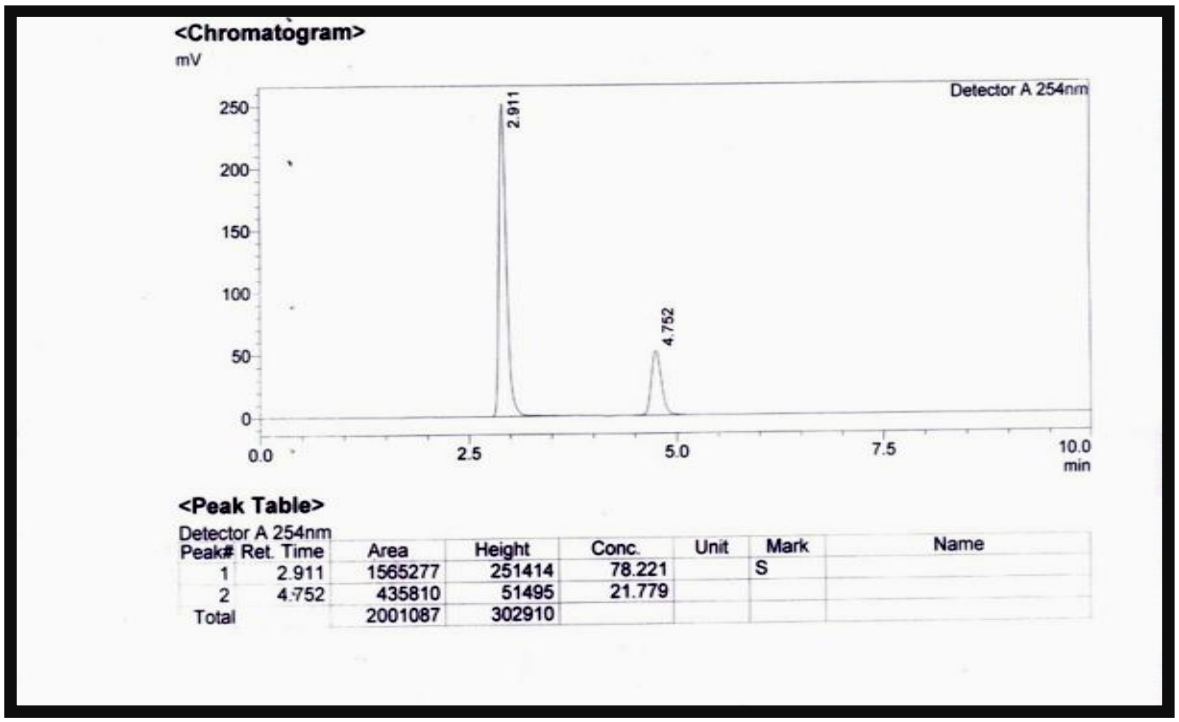


<Peak Table>

Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	2.897	1673557	257051	79.548			
2	4.607	430274	46581	20.452			
Total		2103831	303632				

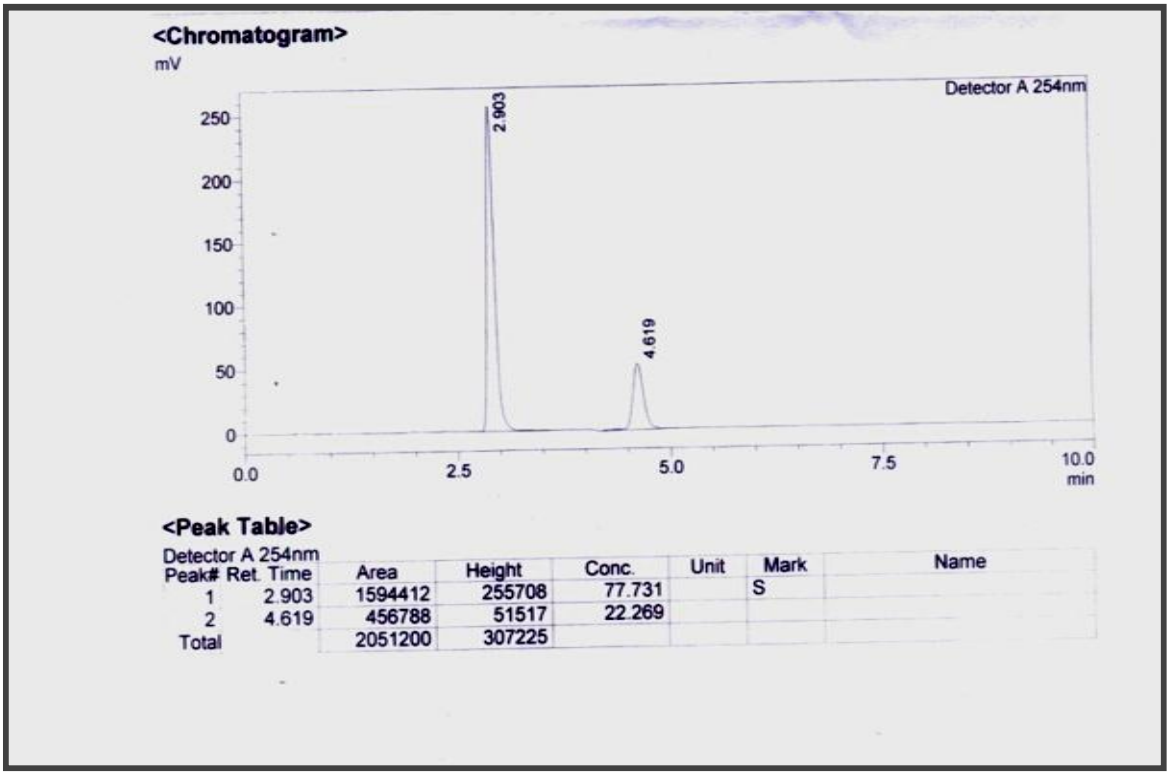
FIG - 25 ANALYSIS OF FORMULATION - 2



<Peak Table>
Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	2.911	1565277	251414	78.221		S	
2	4.752	435810	51495	21.779			
Total		2001087	302910				

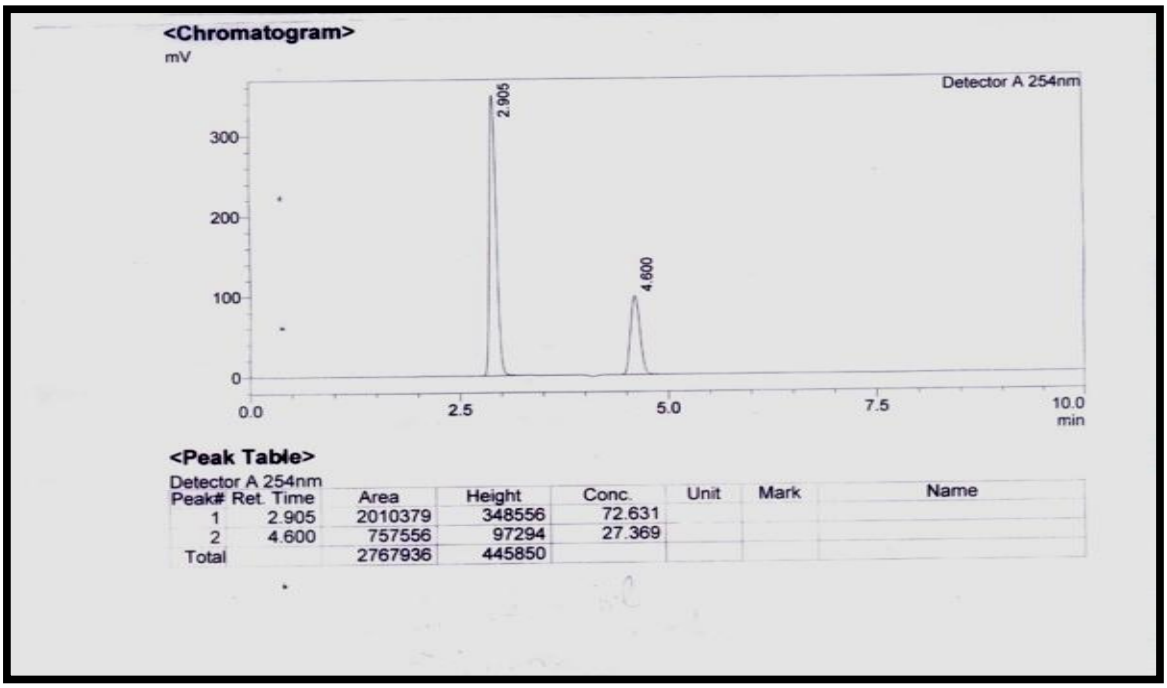
FIG - 26 ANALYSIS OF FORMULATION - 3



<Peak Table>
Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	2.903	1594412	255708	77.731		S	
2	4.619	456788	51517	22.269			
Total		2051200	307225				

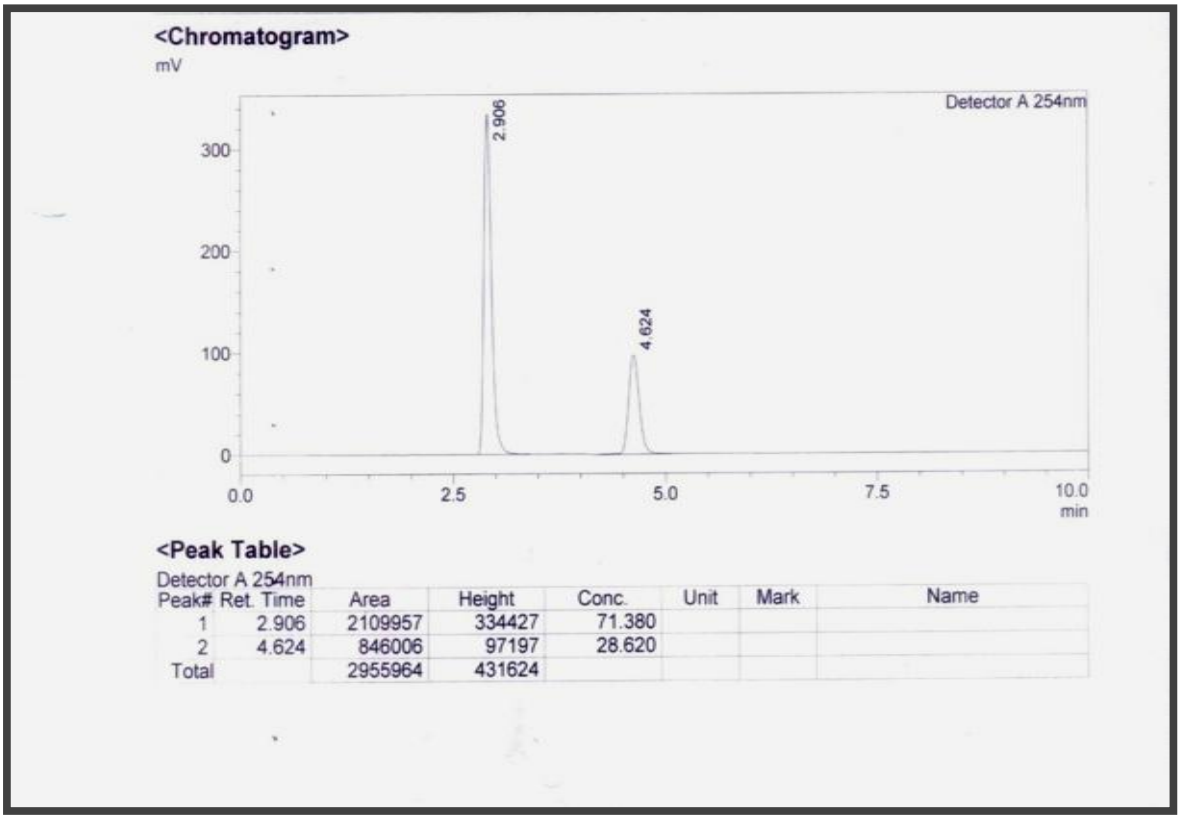
FIG - 27 CHROMATOGRAM FOR 80% RECOVERY ANALYSIS



<Peak Table>
Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	2.905	2010379	348556	72.631			
2	4.600	757556	97294	27.369			
Total		2767936	445850				

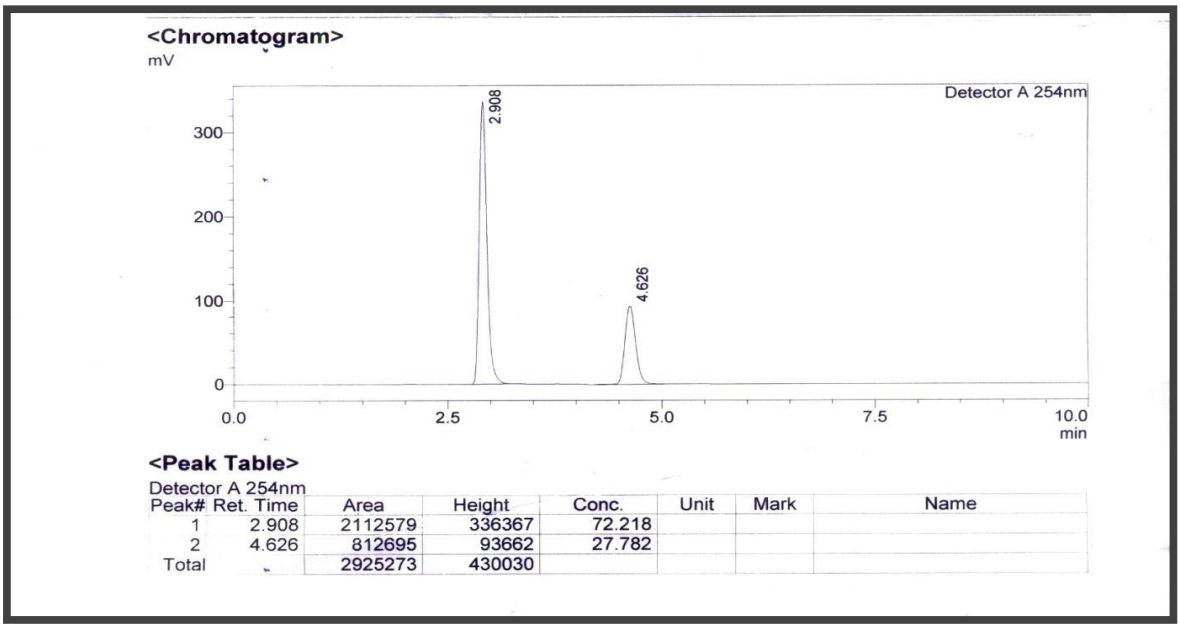
FIG - 28 CHROMATOGRAM FOR 100% RECOVERY ANALYSIS



<Peak Table>
Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	2.906	2109957	334427	71.380			
2	4.624	846006	97197	28.620			
Total		2955964	431624				

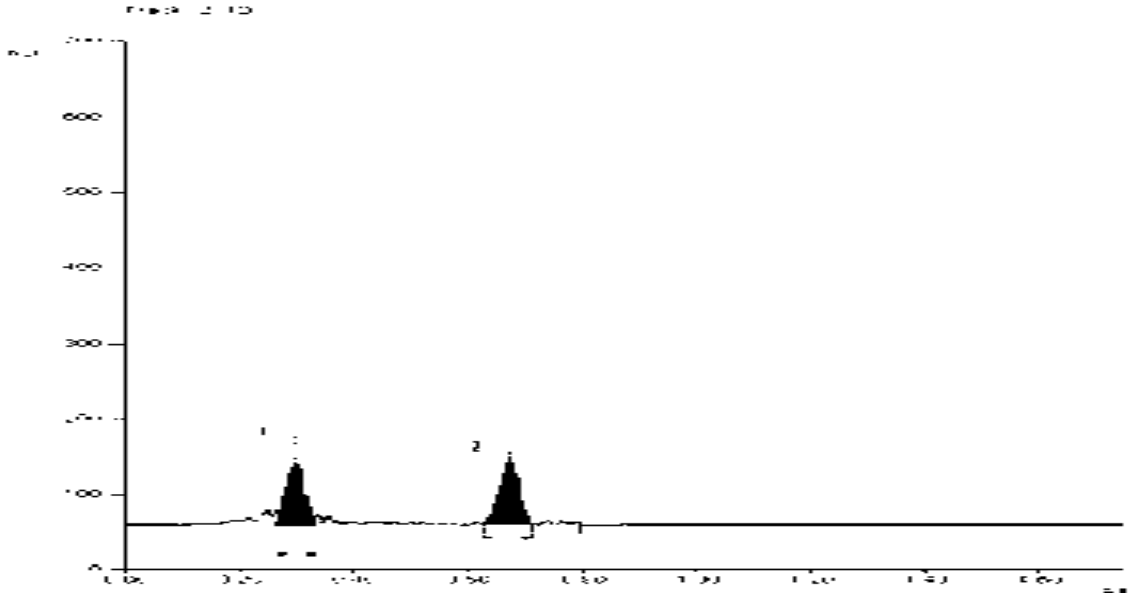
FIG - 29 CHROMATOGRAM FOR 120% RECOVERY ANALYSIS



<Peak Table>
Detector A 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	2.908	2112579	336367	72.218			
2	4.626	812695	93662	27.782			
Total		2925273	430030				

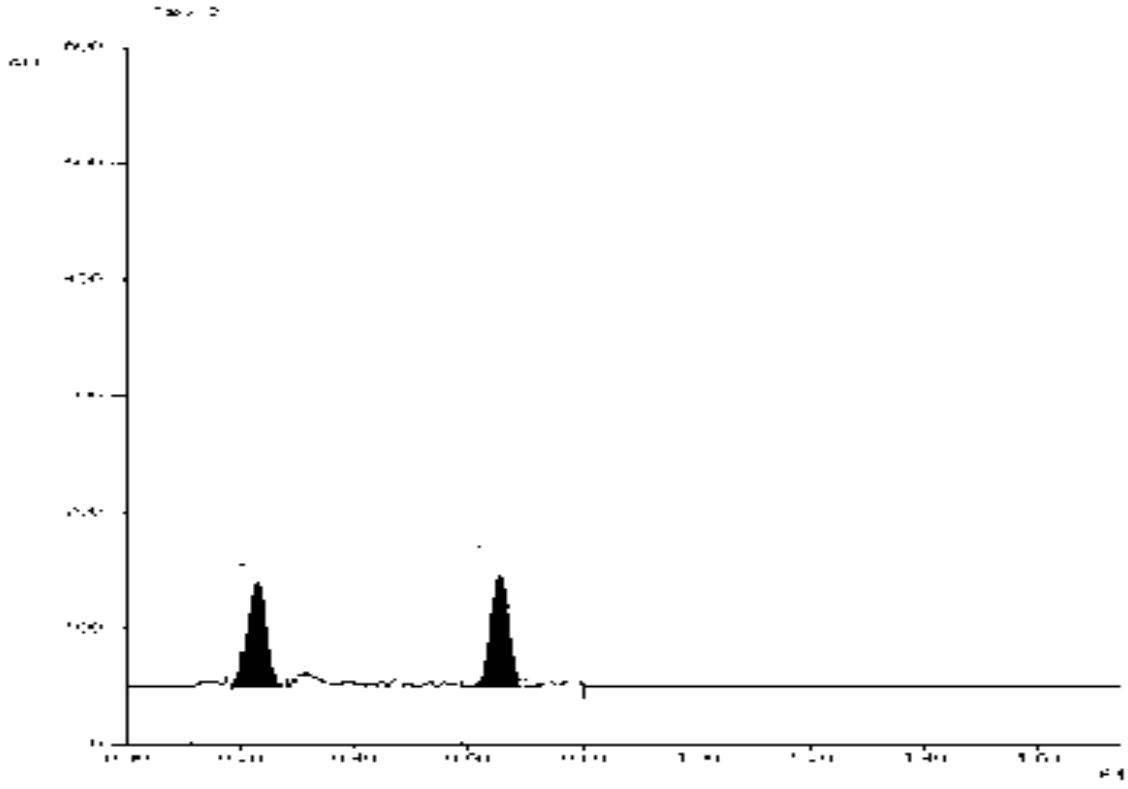
FIG - 30 HPTLC LINEARITY CHROMATOGRAM OF TOLPERISONE HYDROCHLORIDE AND PARACETAMOL (20 ng/µl +40 ng/µl)



RETENTION FACTOR

Peak	Start Rf	Start Height	Max Rf	Max height	Max %	End Rf	End Height	Area	Area %
1	0.24	2.4	0.14	43.3	9.93	0.17	4.4	222.8	9.18
2	0.62	10.6	0.25	16.2	3.72	0.28	11.5	193.8	7.97

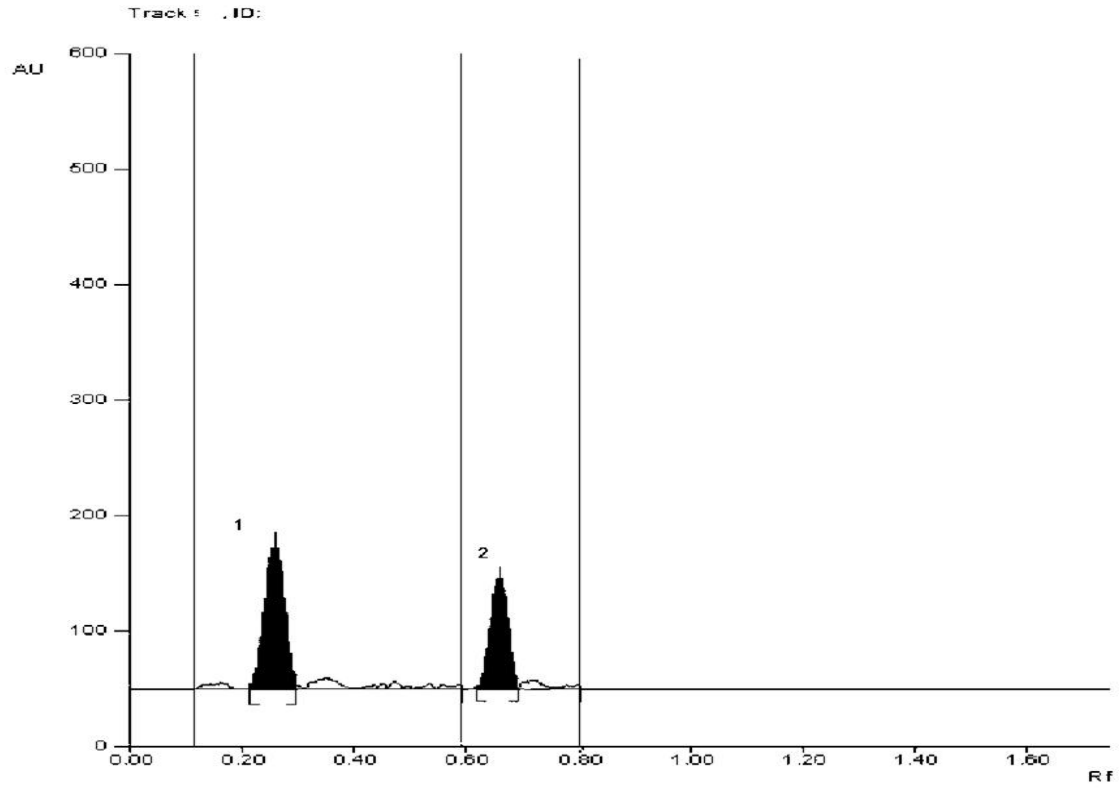
FIG – 31 HPTLC LINEARITY CHROMATOGRAM OF TOLPERISONE HYDROCHLORIDE AND PARACETAMOL (40 ng/µl + 80 ng/µl)



RETENTION FACTOR

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Rf	Area	Area %
1	0.24	2.7	0.14	43.0	6.02	0.16	5.5	456.2	9.41
2	0.64	10.7	0.25	21.3	5.58	0.29	15.0	387.3	8.03

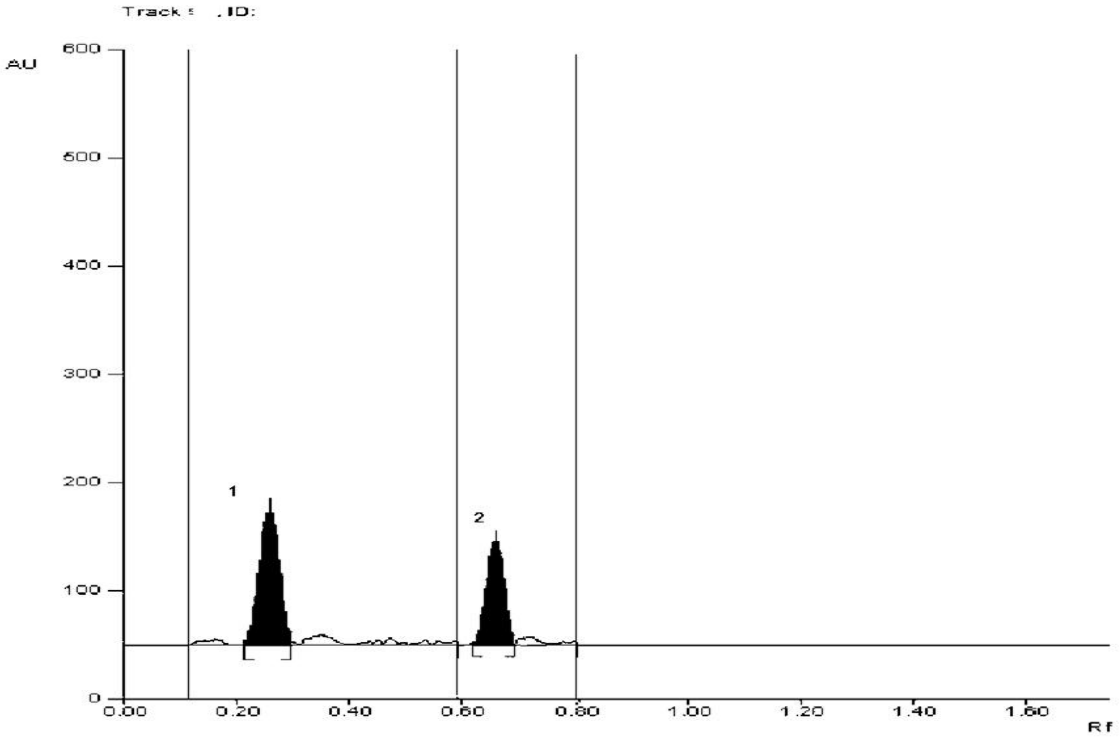
FIG – 32 HPTLC LINEARITY CHROMATOGRAM OF TOLPERISONE HYDROCHLORIDE AND PARACETAMOL (60 ng/µl + 120 ng/µl)



RETENTION FACTOR

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.24	3.3	0.14	44.3	12.36	0.17	6.8	664.2	9.10
2	0.65	13.1	0.25	27.4	7.63	0.27	18.9	589.2	8.07

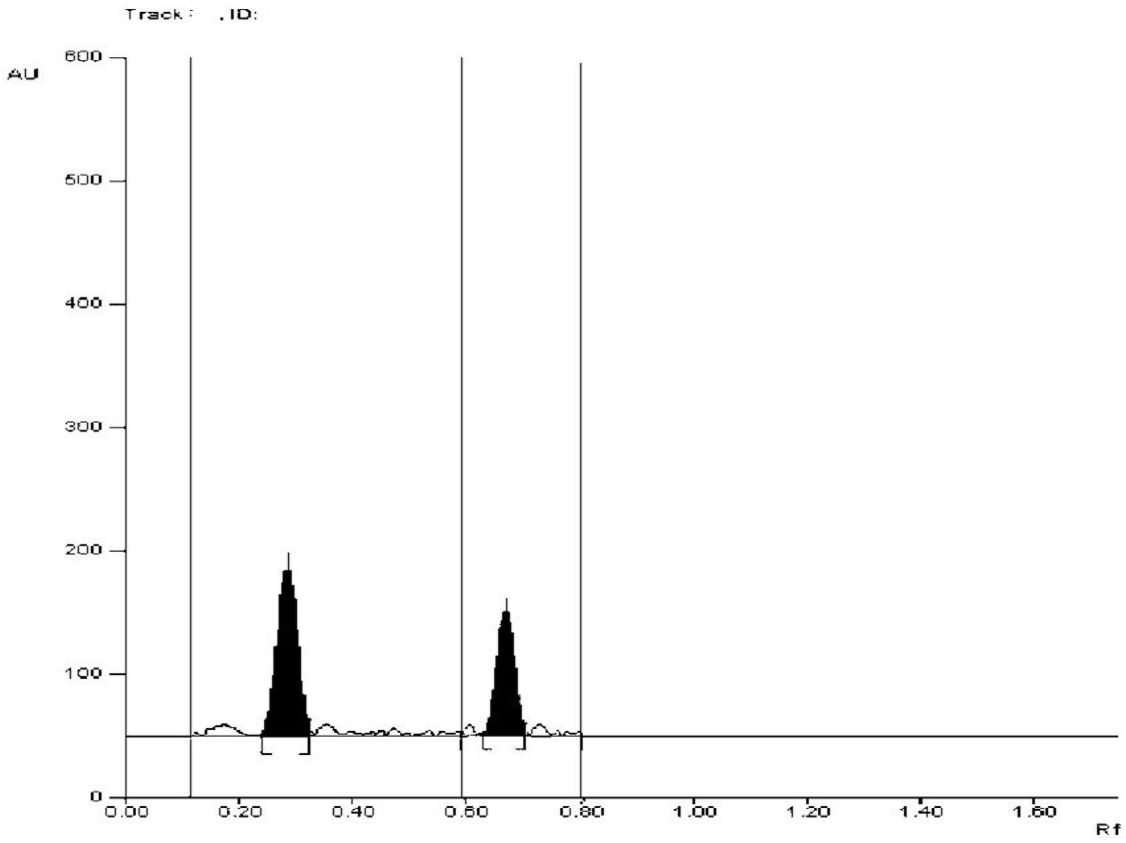
FIG - 33 HPTLC LINEARITY CHROMATOGRAM OF TOLPERISONE HYDROCHLORIDE AND PARACETAMOL (80 ng/µl +160 ng/µl)



RETENTION FACTOR

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.24	2.3	0.14	60.6	16.84	0.16	3.9	889.2	9.18
2	0.64	11.5	0.25	33.6	9.35	0.27	19.5	773.8	8.05

FIG - 34 HPTLC LINEARITY CHROMATOGRAM OF TOLPERISONE HYDROCHLORIDE AND PARACETAMOL (100 ng/µl +200ng/µl)



RETENTION FACTOR

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.24	2.7	0.14	76.1	29.41	0.16	2.1	1121.4	9.05
2	0.65	6.8	0.25	36.0	13.91	0.27	15.1	960.4	7.90

FIG – 35 CALIBRATION CURVE OF TOLPERISONE HYDROCHLORIDE BY HPTLC METHOD

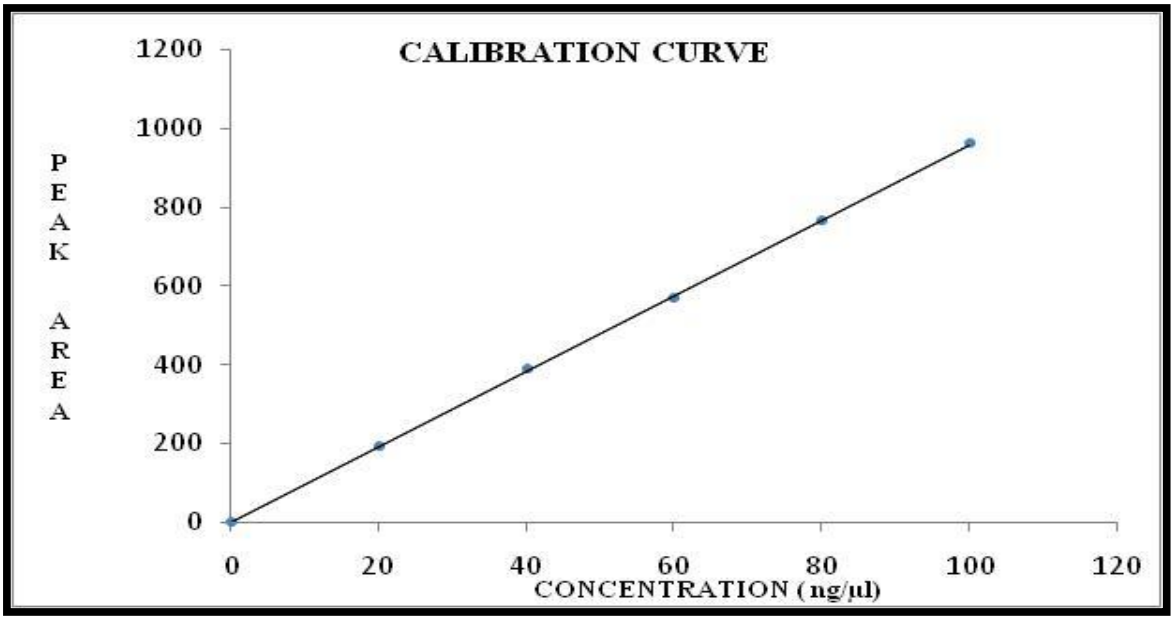


FIG – 36 CALIBRATION CURVE OF PARACETAMOL BY HPTLC METHOD

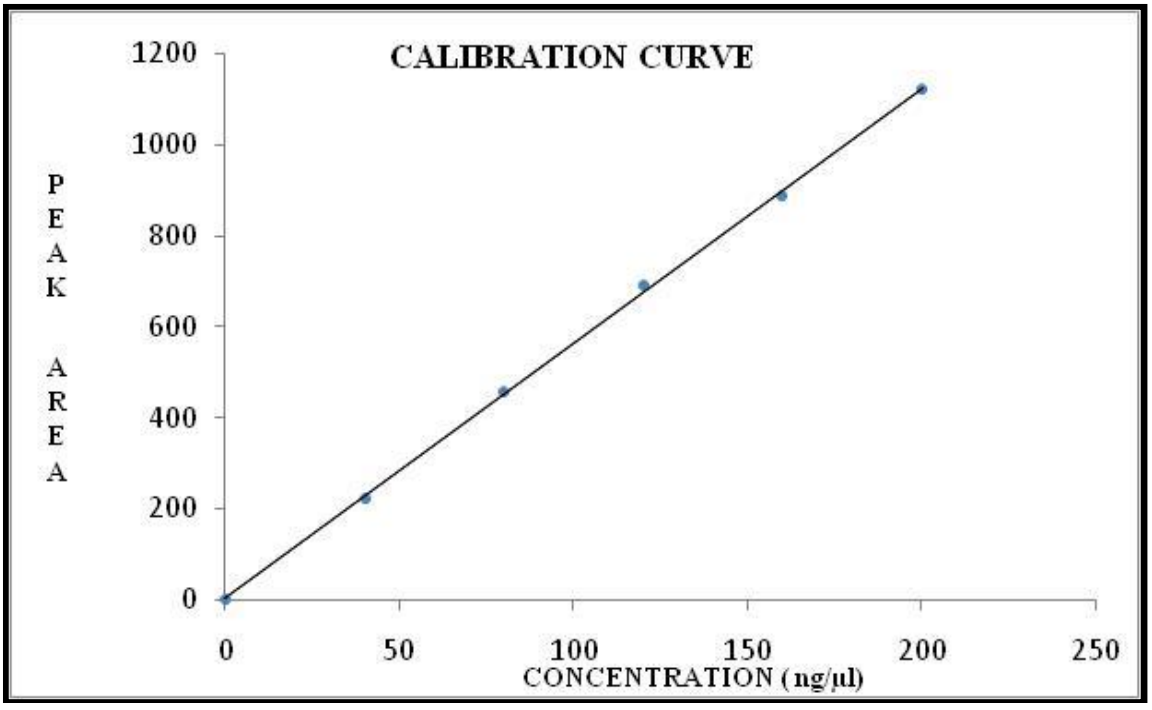
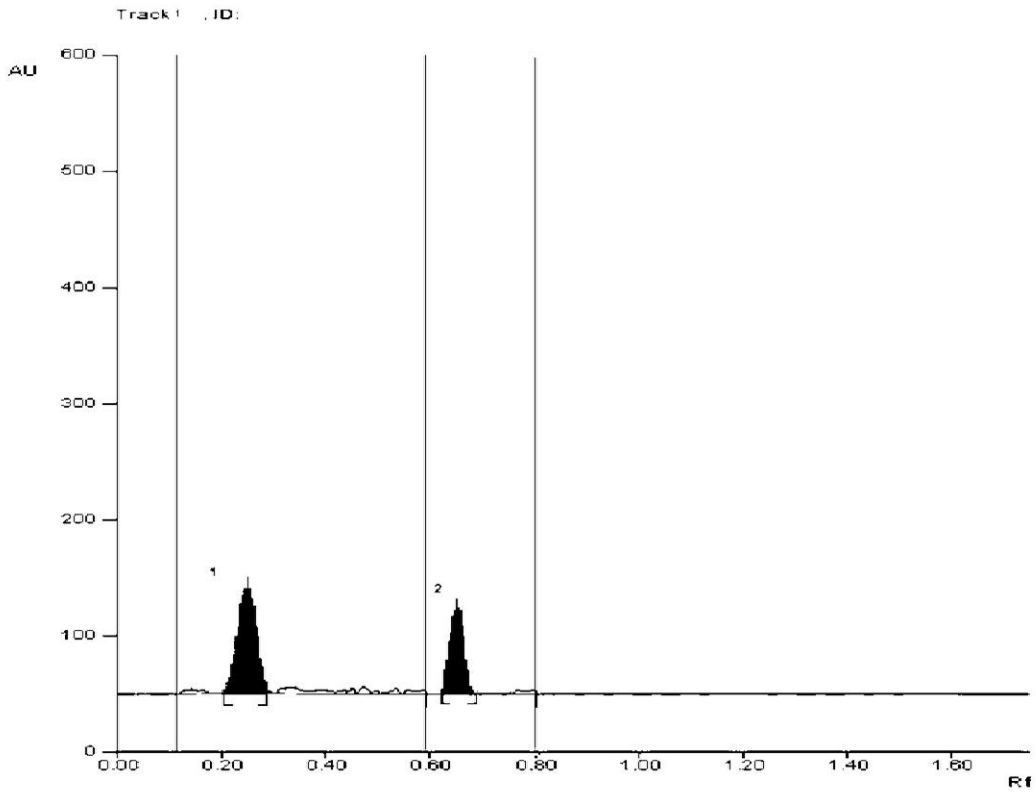


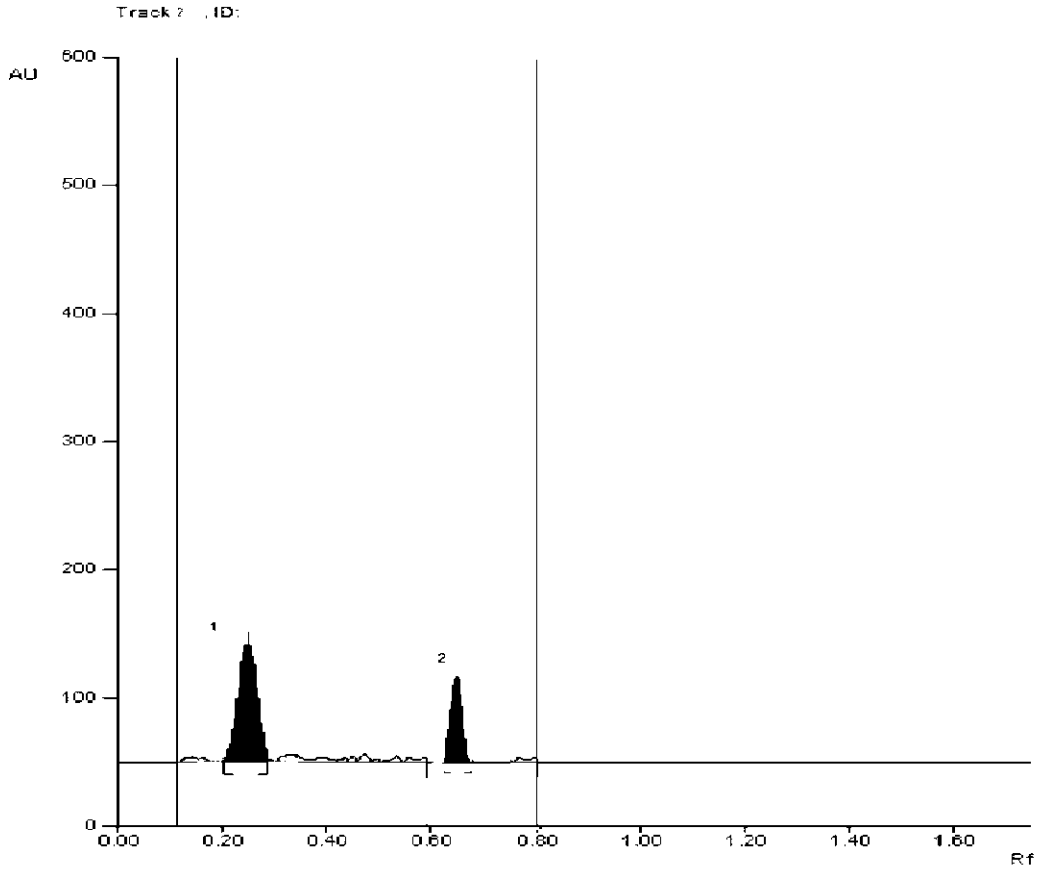
FIG - 37 ANALYSIS OF FORMULATION - 1



RETENTION FACTOR

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.24	7.8	0.15	16.9	3.23	0.17	12.3	670	9.40
2	0.64	15.7	0.25	27.4	5.25	0.27	13.6	598	7.88

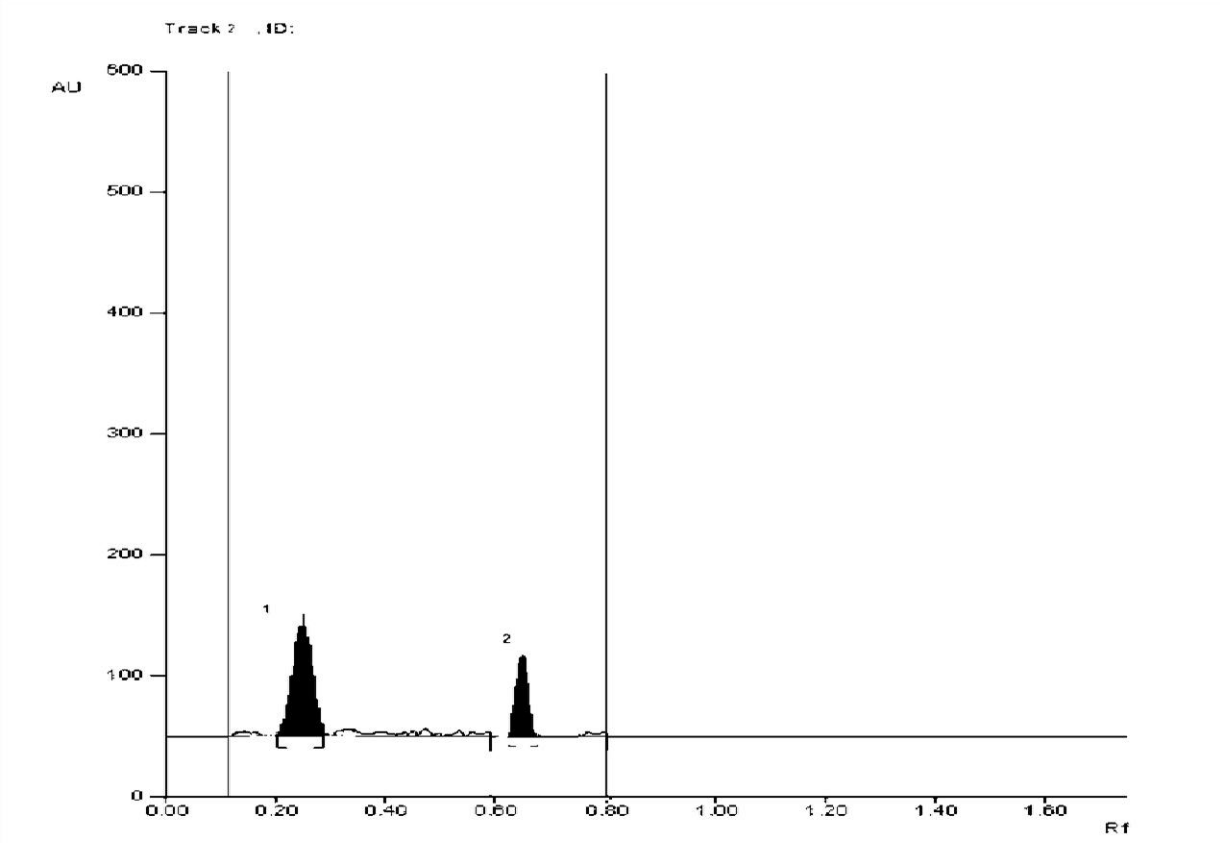
FIG – 38 ANALYSIS OF FORMULATION - 2



RETENTION FACTOR

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.25	7.6	0.15	16.7	3.29	0.17	12.3	678	9.34
2	0.65	15.6	0.25	27.5	5.25	0.27	13.4	568.0	7.96

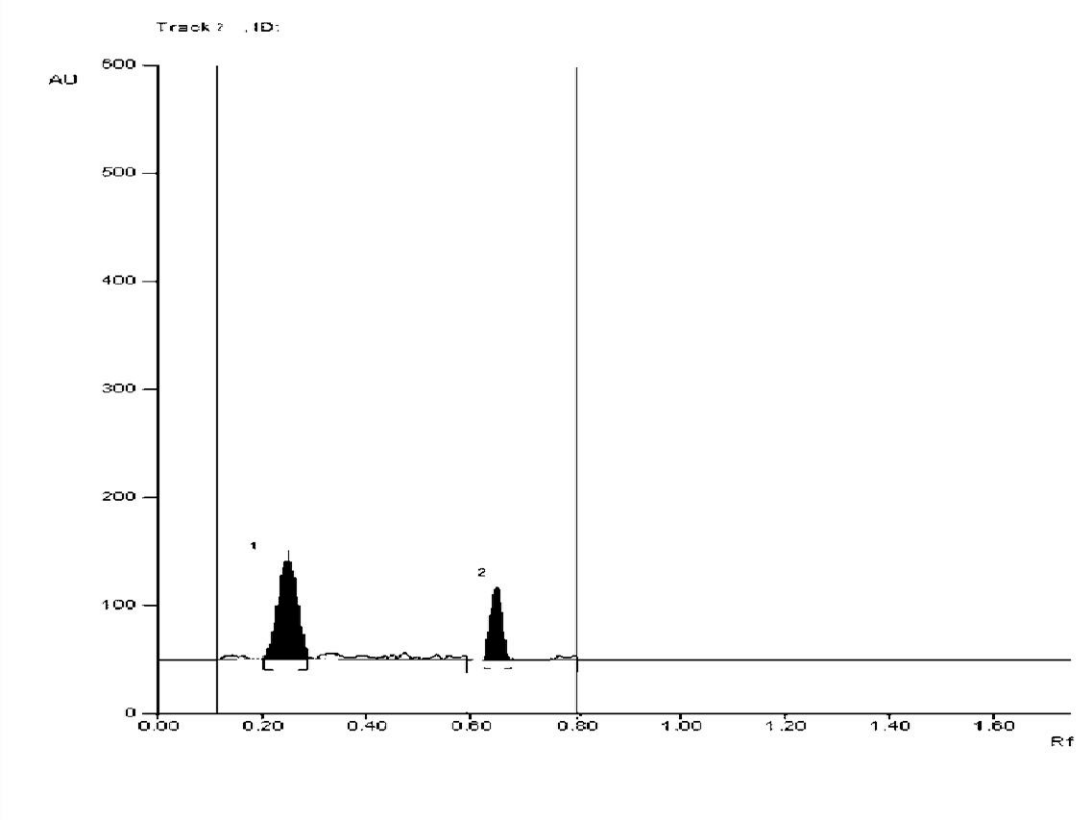
FIG – 39 ANALYSIS OF FORMULATION - 3



RETENTION FACTOR

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.25	7.6	0.15	16.7	3.29	0.17	12.3	673	9.34
2	0.65	15.6	0.25	27.5	5.25	0.27	13.4	579	7.96

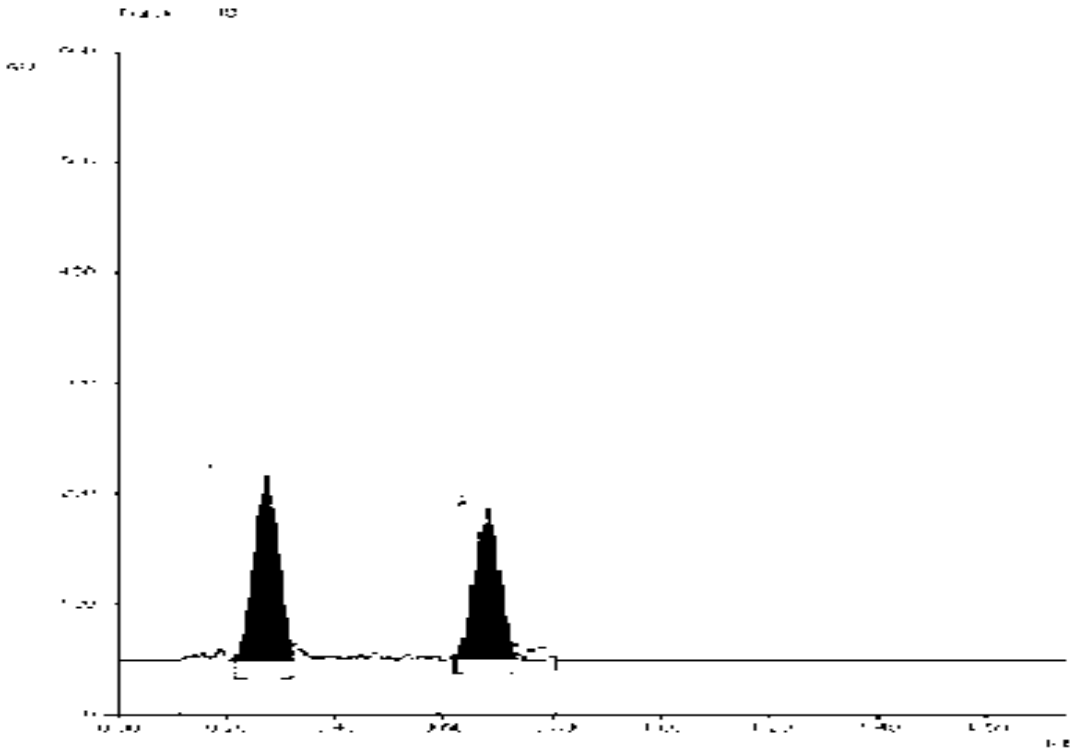
FIG – 40 RECOVERY ANALYSIS OF 80% FORMULATION



RETENTION FACTOR

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.25	7.6	0.15	16.7	3.29	0.17	12.3	798	9.34
2	0.65	15.6	0.25	27.5	5.25	0.27	13.4	685	7.96

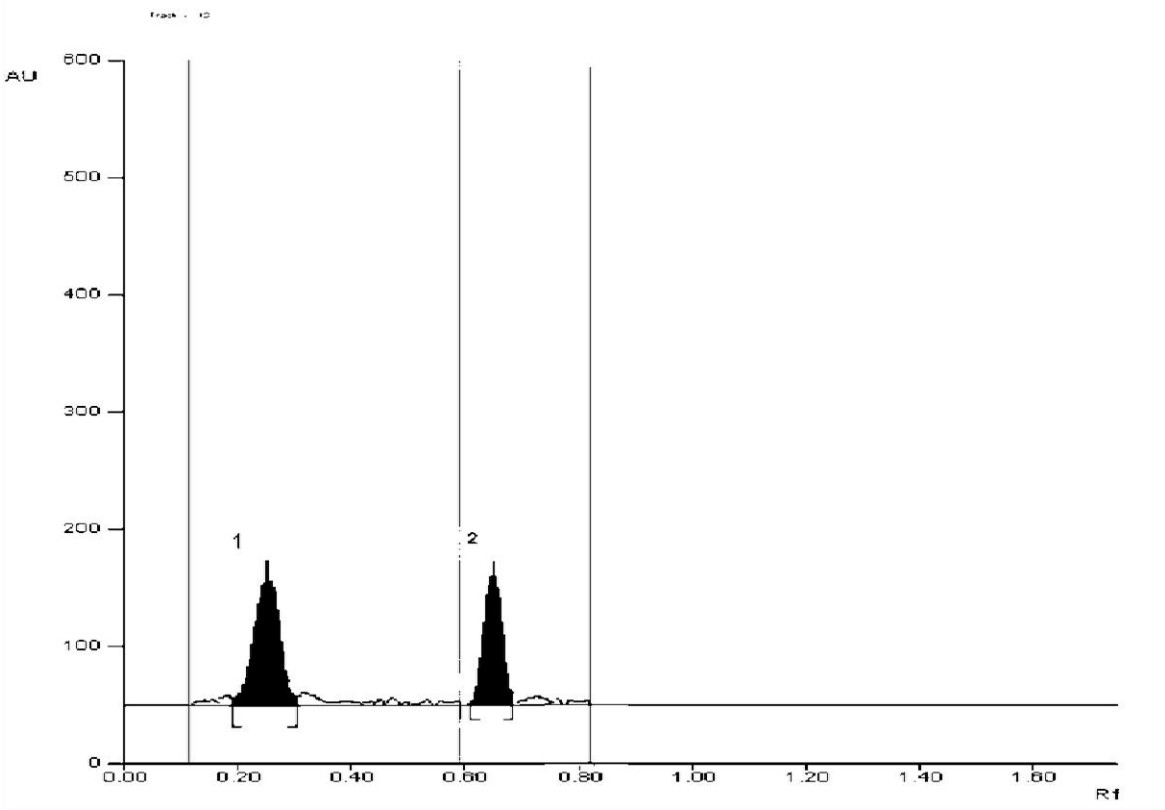
FIG - 41 RECOVERY ANALYSIS OF 100% FORMULATION



RETENTION FACTOR

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.25	7.6	0.15	16.7	3.29	0.17	12.3	859	9.34
2	0.65	15.6	0.25	27.5	5.25	0.27	13.4	764	7.96

FIG – 42 RECOVERY ANALYSIS OF 120% FORMULATION



RETENTION FACTOR

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.13	11.9	0.15	57.0	8.52	0.18	15.8	446.4	9.43
2	0.23	22.1	0.25	34.3	5.13	0.28	23.7	393.2	7.96

FIG - 43 IR SPECTRA OF SITAGLIPTIN PHOSPHATE

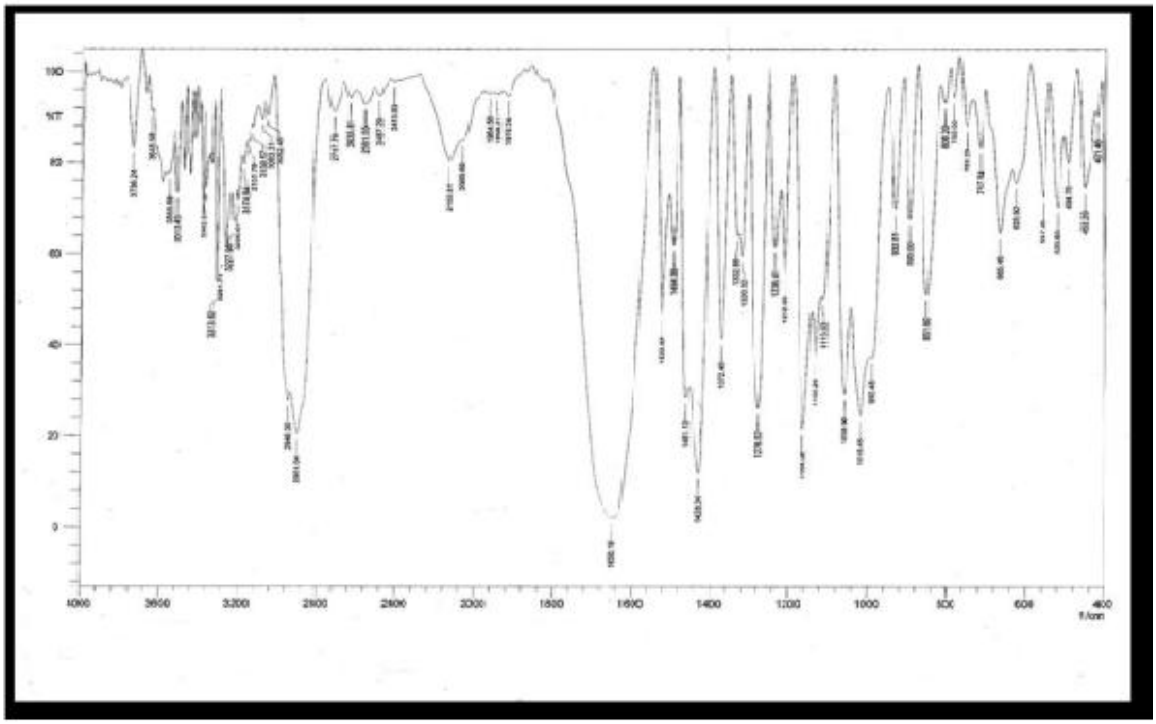


FIG – 44 IR SPECTRA OF SIMVASTATIN

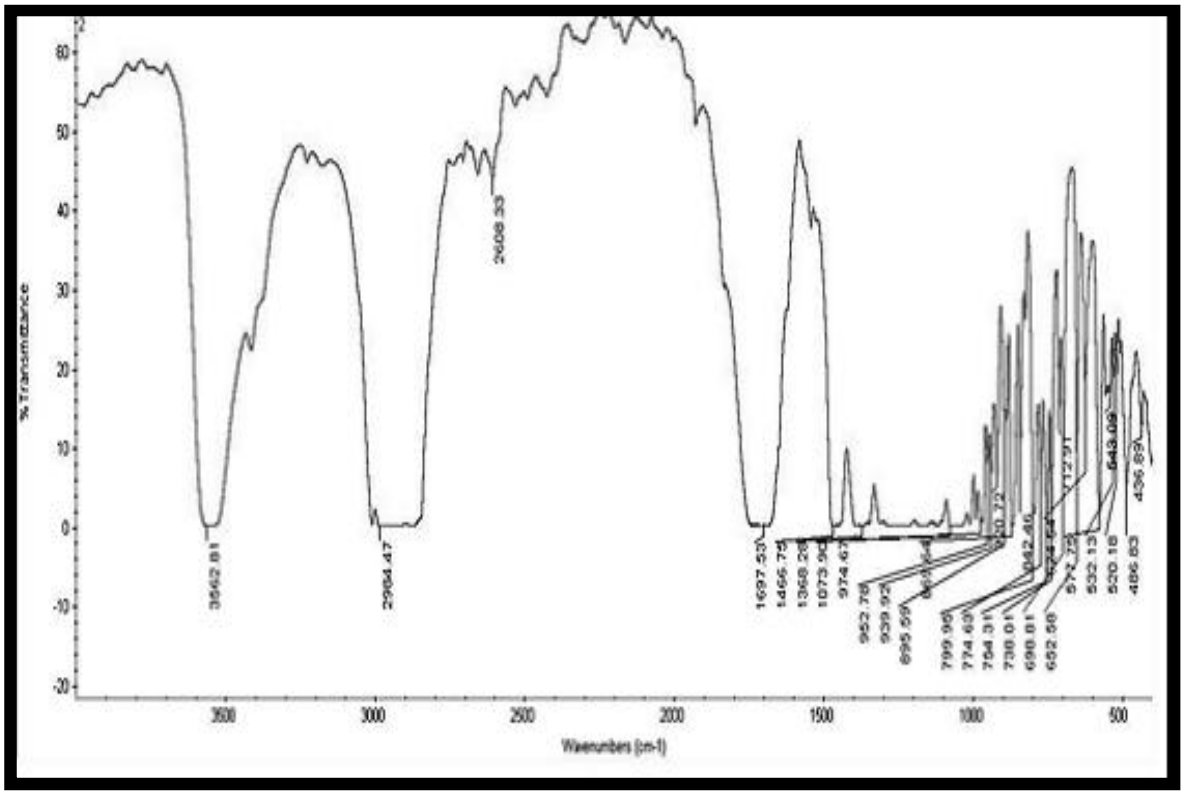


FIG – 45 OVERLAIN SPECTRA OF SIMVASTATIN AND SITAGLIPTIN PHOSPHATE

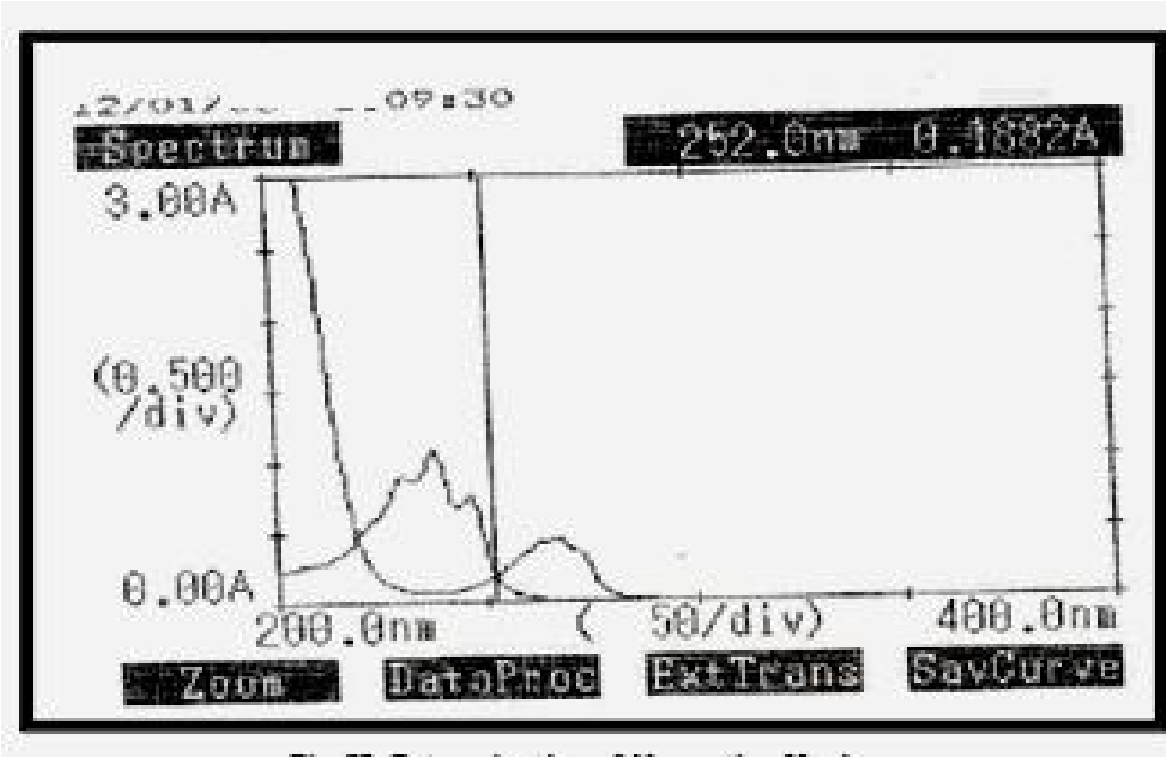


FIG – 46 FIRST ORDER DERIVATIVE SPECTRA OF SITAGLIPTIN PHOSPHATE AND SIMVASTATIN

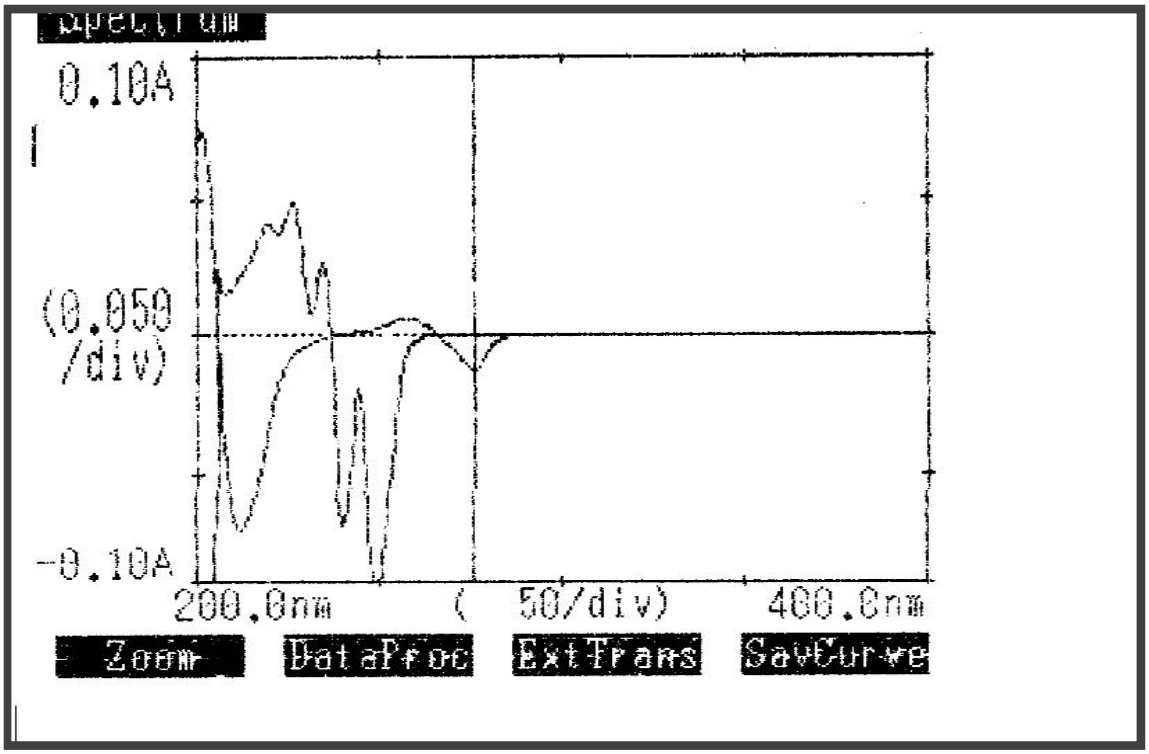


FIG - 47 CALIBRATION CURVE OF SITAGLIPTIN PHOSPHATE (FIRST ORDER DERIVATIVE METHOD)

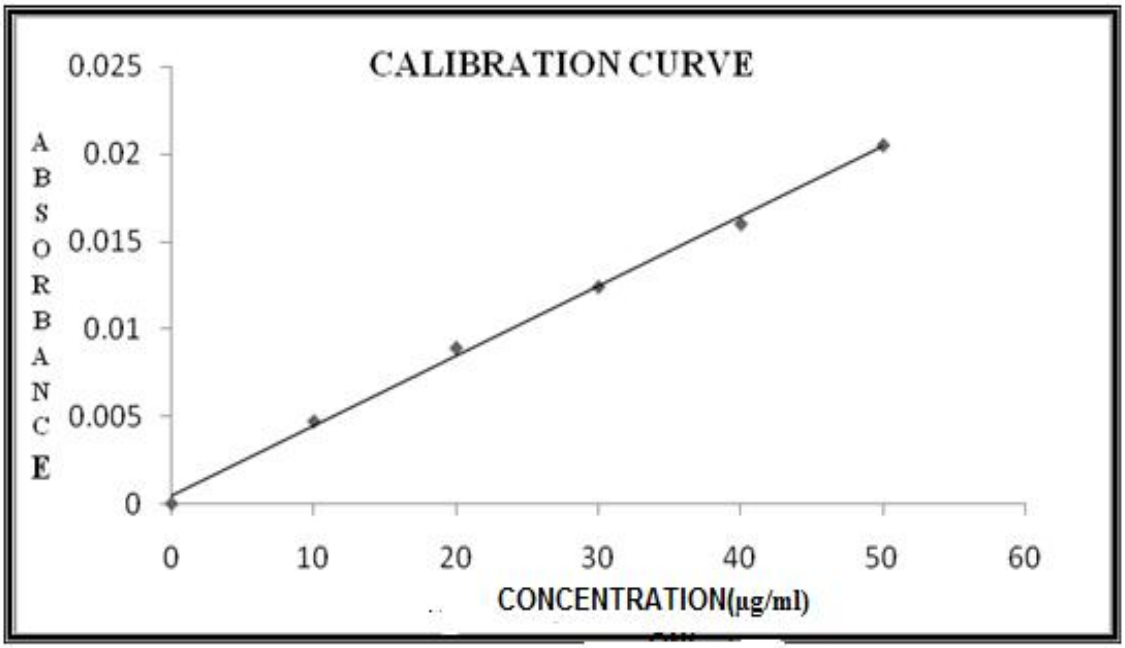


FIG – 48 CALIBRATION CURVE OF SIMVASTATIN (FIRST ORDER DERIVATIVE METHOD)

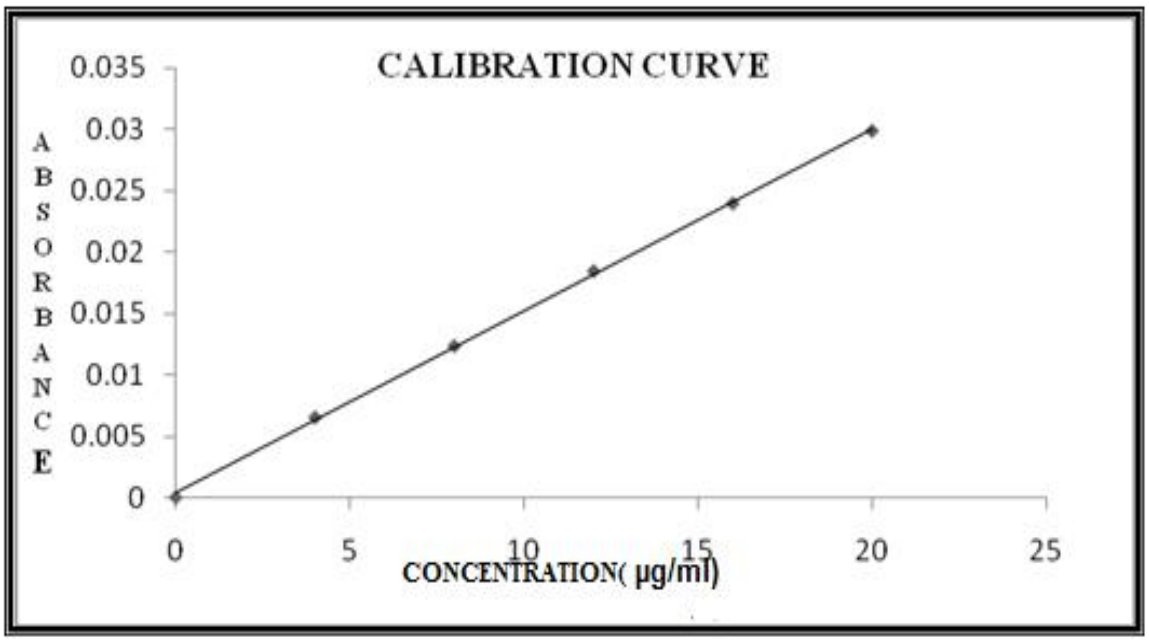
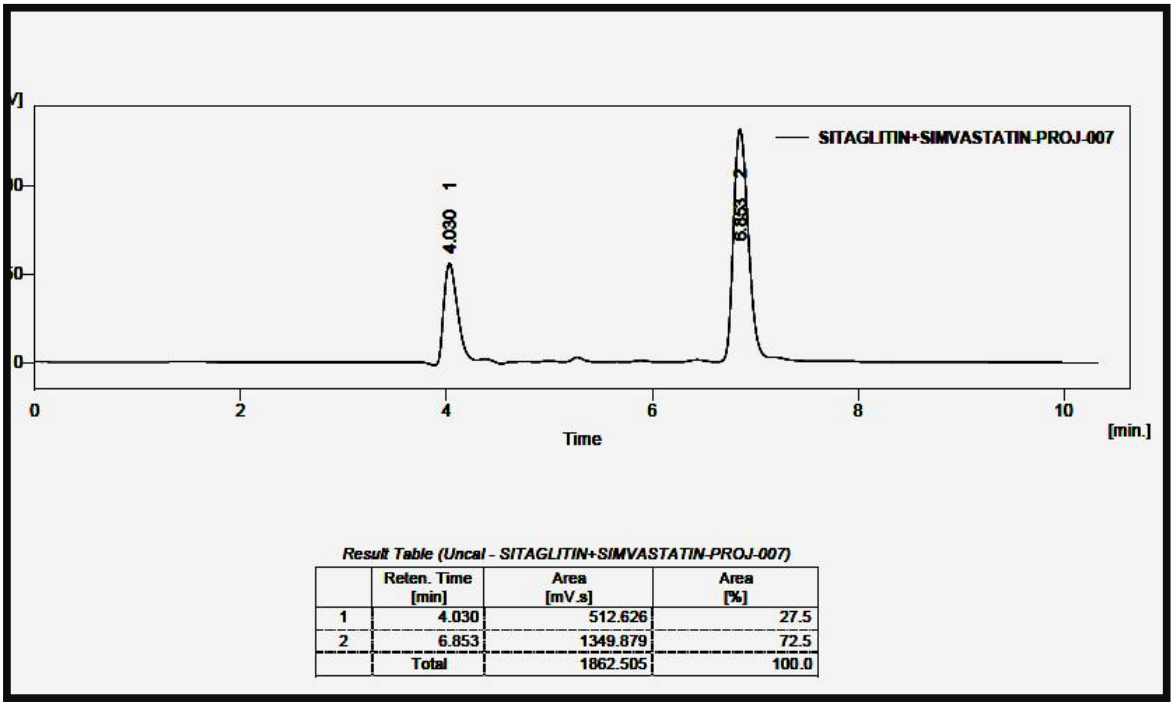


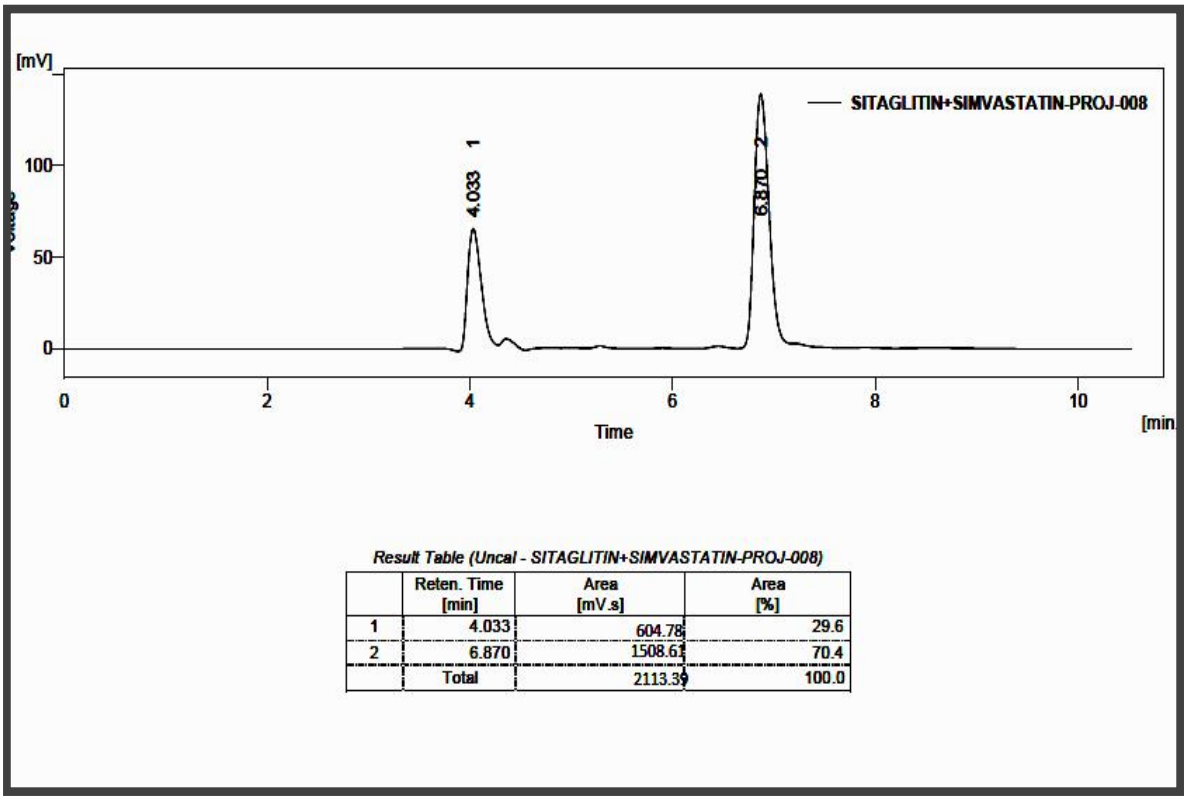
FIG -- 49 RP- HPLC LINEARITY CHROMATOGRAM OF SITAGLIPTIN PHOSPHATE AND SIMVASTATIN (160, 64 µg/ ml)



Result Table (Uncal - SITAGLITIN+SIMVASTATIN-PROJ-007)

	Reten. Time [min]	Area [mV.s]	Area [%]
1	4.030	512.626	27.5
2	6.853	1349.879	72.5
	Total	1862.505	100.0

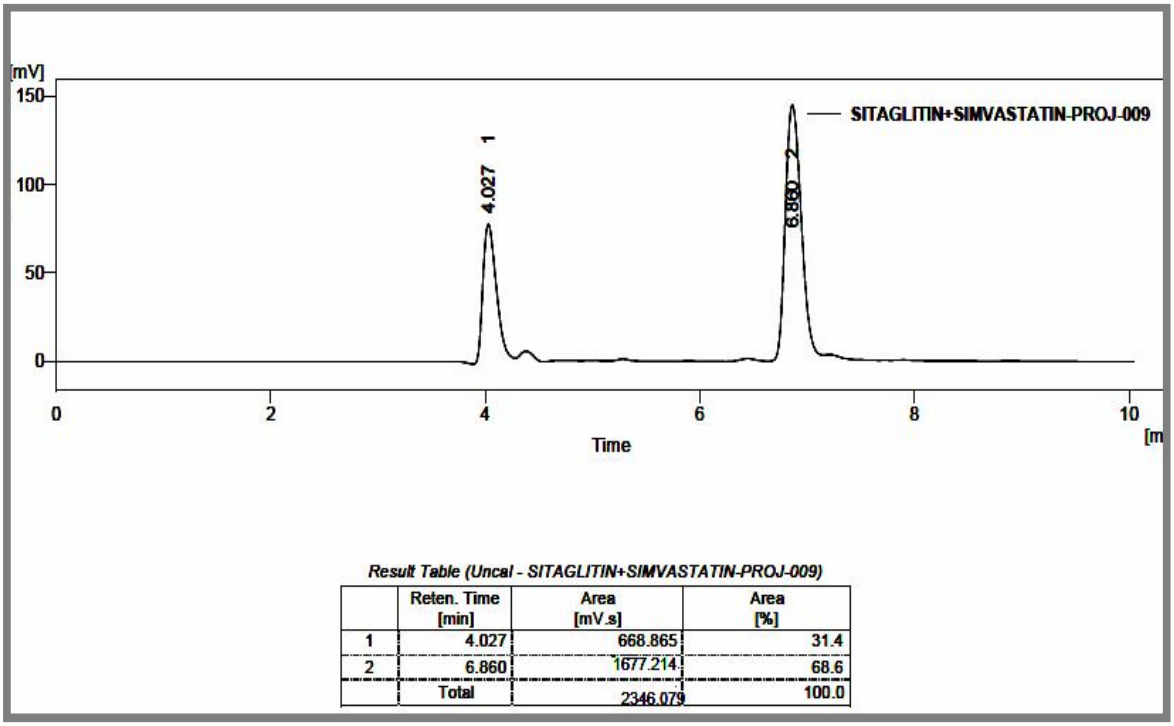
FIG – 50 RP- HPLC LINEARITY CHROMATOGRAM OF SITAGLIPTIN PHOSPHATE AND SIMVASTATIN (180, 72 µg/ ml)



Result Table (Uncal - SITAGLITIN+SIMVASTATIN-PROJ-008)

	Reten. Time [min]	Area [mV.s]	Area [%]
1	4.033	604.78	29.6
2	6.870	1508.61	70.4
	Total	2113.39	100.0

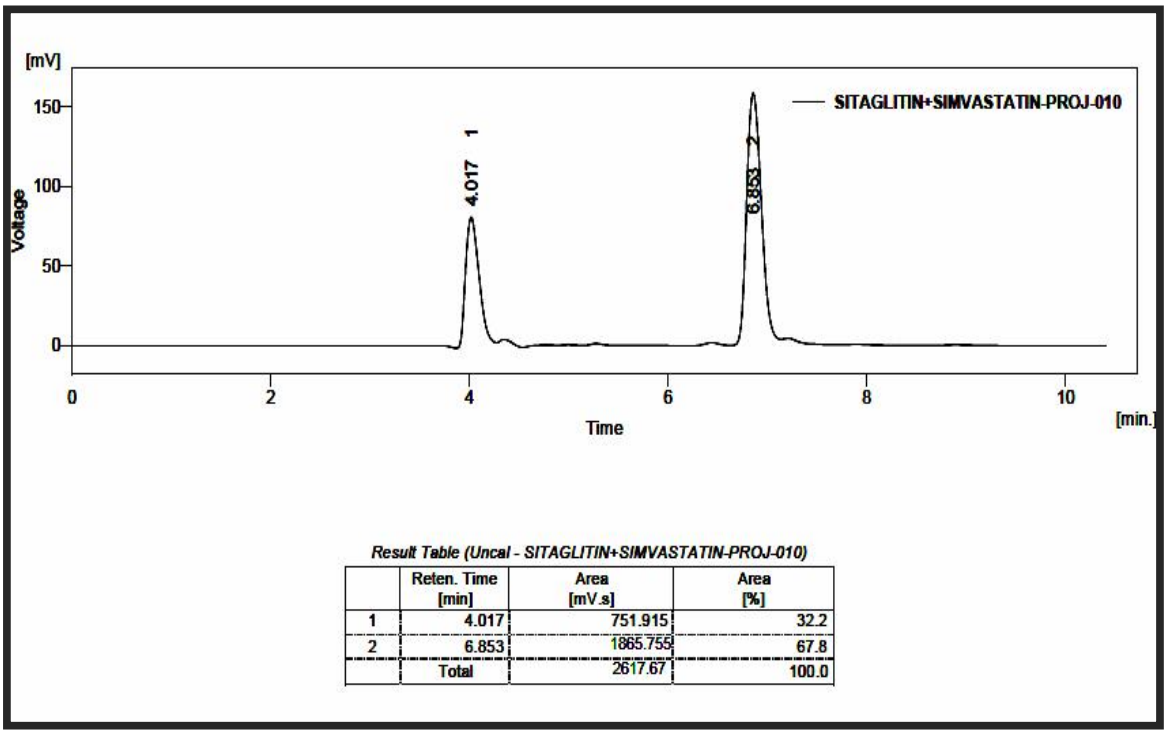
FIG – 51 RP- HPLC LINEARITY CHROMATOGRAM OF SITAGLIPTIN PHOSPHATE AND SIMVASTATIN (200, 80 µg/ ml)



Result Table (Uncal - SITAGLITIN+SIMVASTATIN-PROJ-009)

	Reten. Time [min]	Area [mV.s]	Area [%]
1	4.027	668.865	31.4
2	6.860	1677.214	68.6
	Total	2346.079	100.0

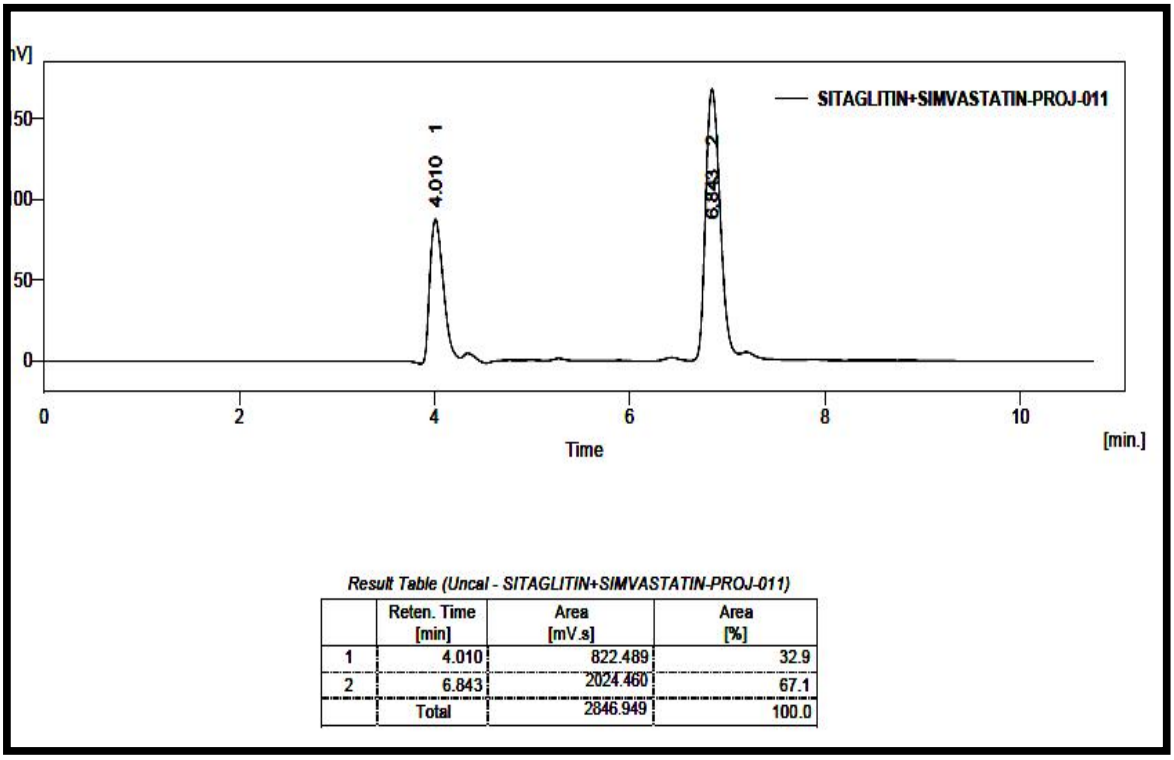
FIG – 52 RP-HPLC LINEARITY CHROMATOGRAM OF SITAGLIPTIN PHOSPHATE AND SIMVASTATIN (220, 88 µg/ml)



Result Table (Uncal - SITAGLITIN+SIMVASTATIN-PROJ-010)

	Reten. Time [min]	Area [mV.s]	Area [%]
1	4.017	751.915	32.2
2	6.853	1865.755	67.8
	Total	2617.67	100.0

FIG – 53 RP-HPLC LINEARITY CHROMATOGRAM OF SITAGLIPTIN PHOSPHATE AND SIMVASTATIN (240, 96 µg/ml)



Result Table (Uncal - SITAGLITIN+SIMVASTATIN-PROJ-011)

	Reten. Time [min]	Area [mV.s]	Area [%]
1	4.010	822.489	32.9
2	6.843	2024.460	67.1
	Total	2846.949	100.0

FIG - 54 CALIBRATION CURVE OF SITAGLIPTIN PHOSPHATE BY RP-HPLC METHOD

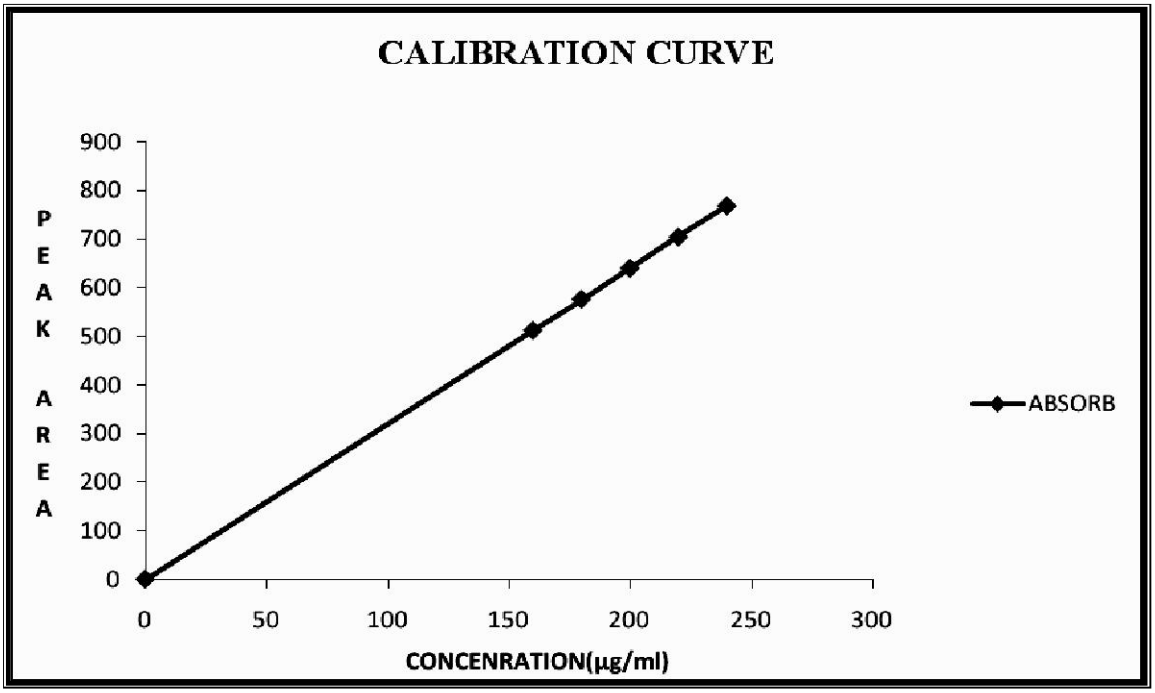


FIG- 55 - CALIBRATION CURVE OF SIMVASTATIN BY RP-HPLC METHOD

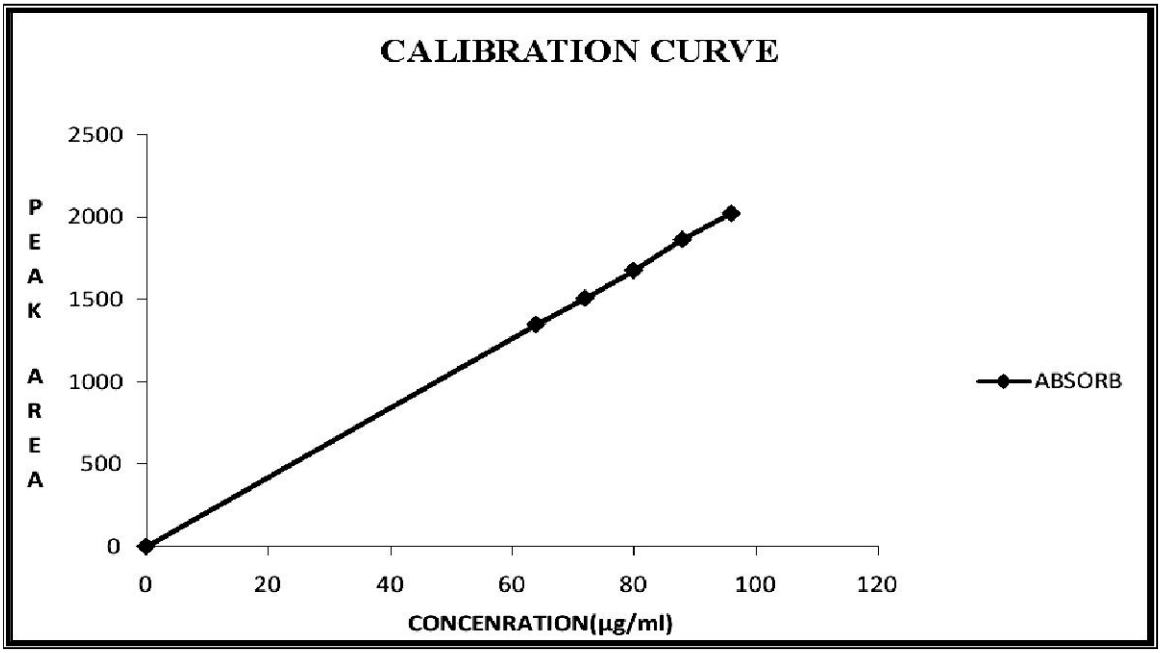
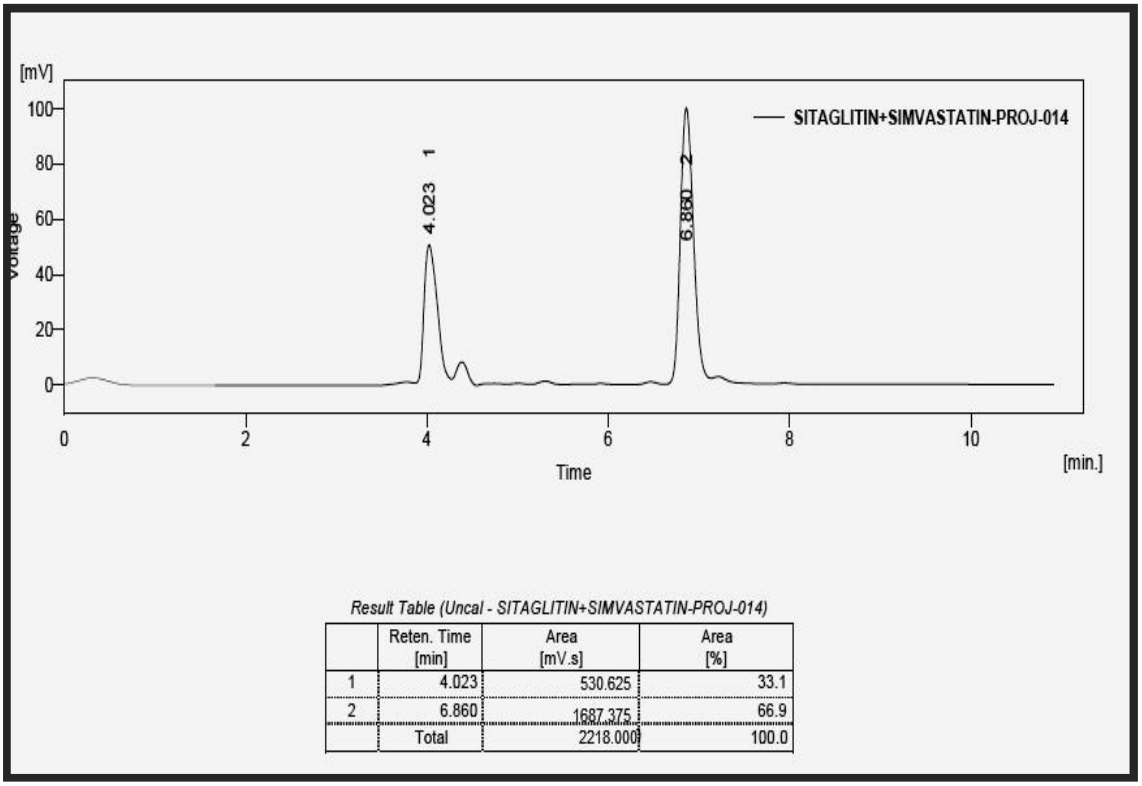


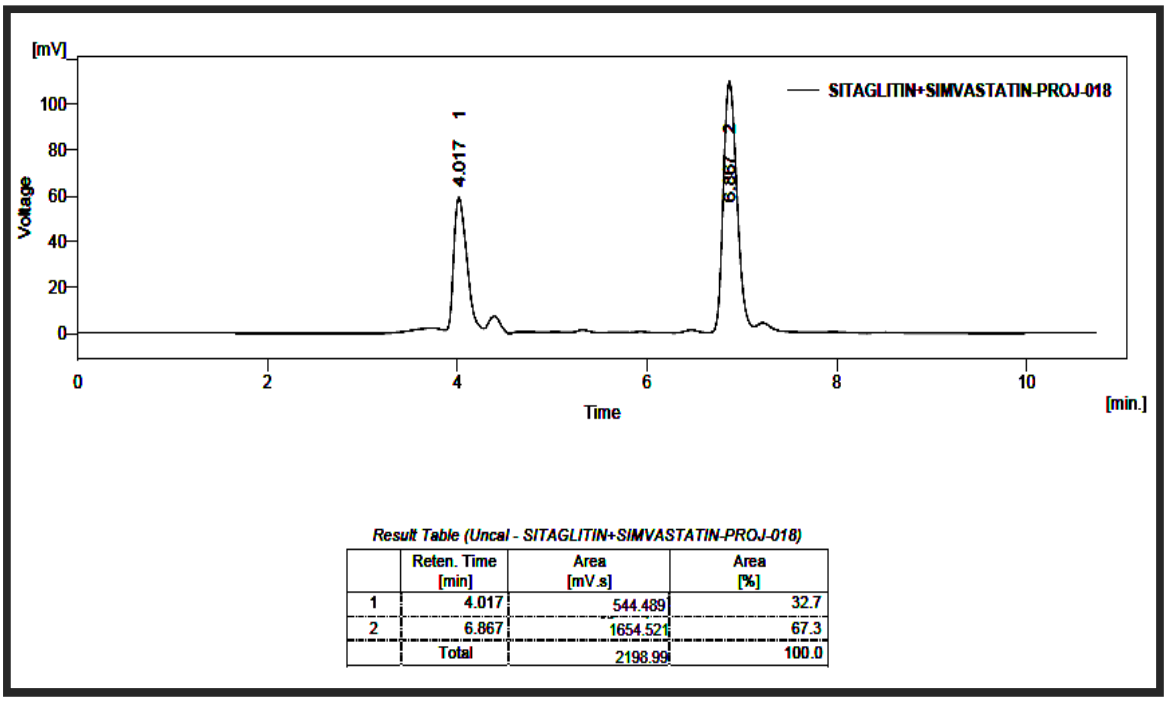
FIG – 56 CHROMATOGRAM FOR FORMULATION -1



Result Table (Uncal - SITAGLITIN+SIMVASTATIN-PROJ-014)

	Reten. Time [min]	Area [mV.s]	Area [%]
1	4.023	530.625	33.1
2	6.860	1687.375	66.9
	Total	2218.000	100.0

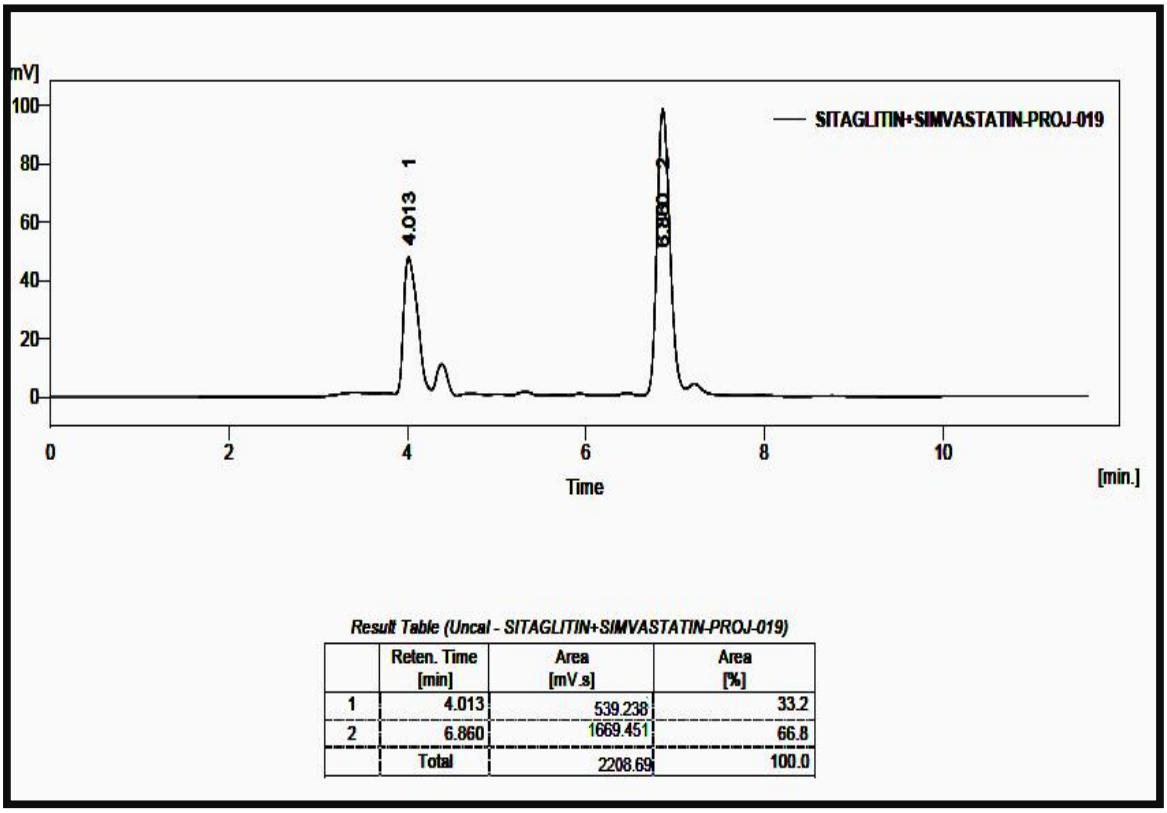
FIG – 57 CHROMATOGRAM FOR FORMULATION – 2



Result Table (Uncal - SITAGLITIN+SIMVASTATIN-PROJ-018)

	Reten. Time [min]	Area [mV.s]	Area [%]
1	4.017	544.489	32.7
2	6.867	1654.521	67.3
	Total	2198.99	100.0

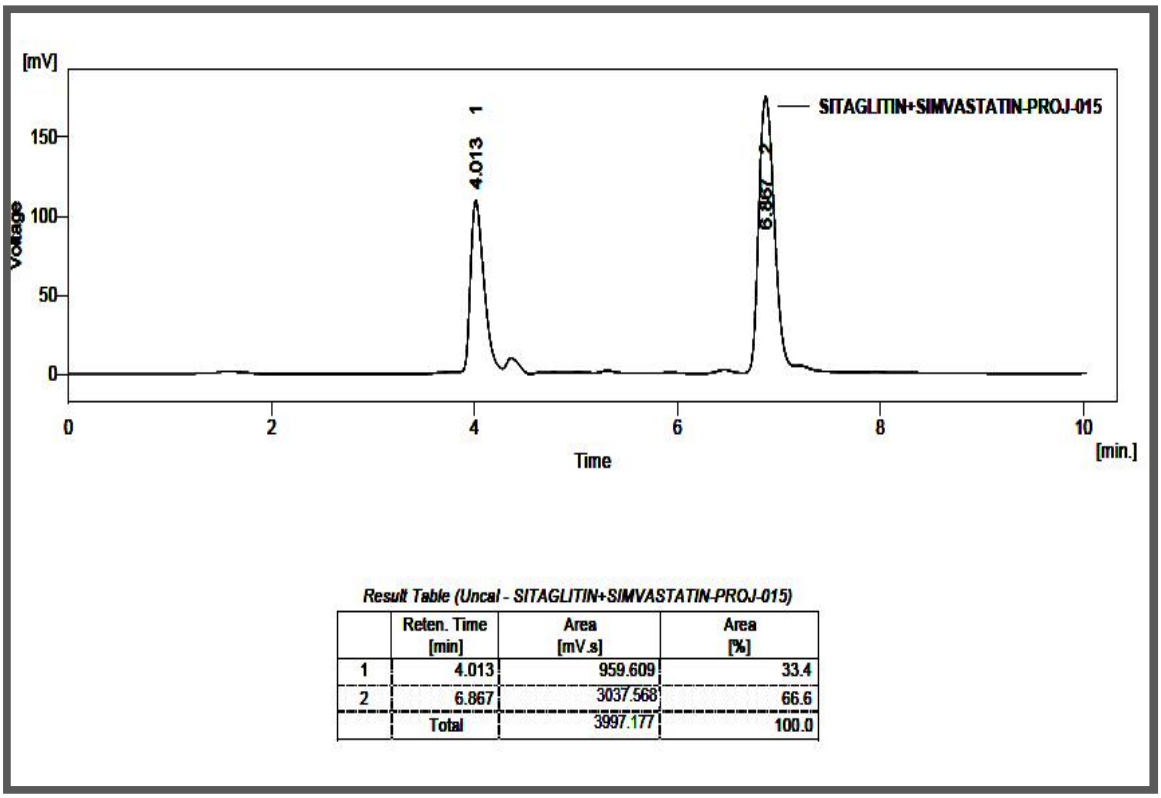
FIG – 58 CHROMATOGRAM FOR FORMULATION – 3



Result Table (Uncal - SITAGLITIN+SIMVASTATIN-PROJ-019)

	Reten. Time [min]	Area [mV.s]	Area [%]
1	4.013	539.238	33.2
2	6.860	1669.451	66.8
	Total	2208.69	100.0

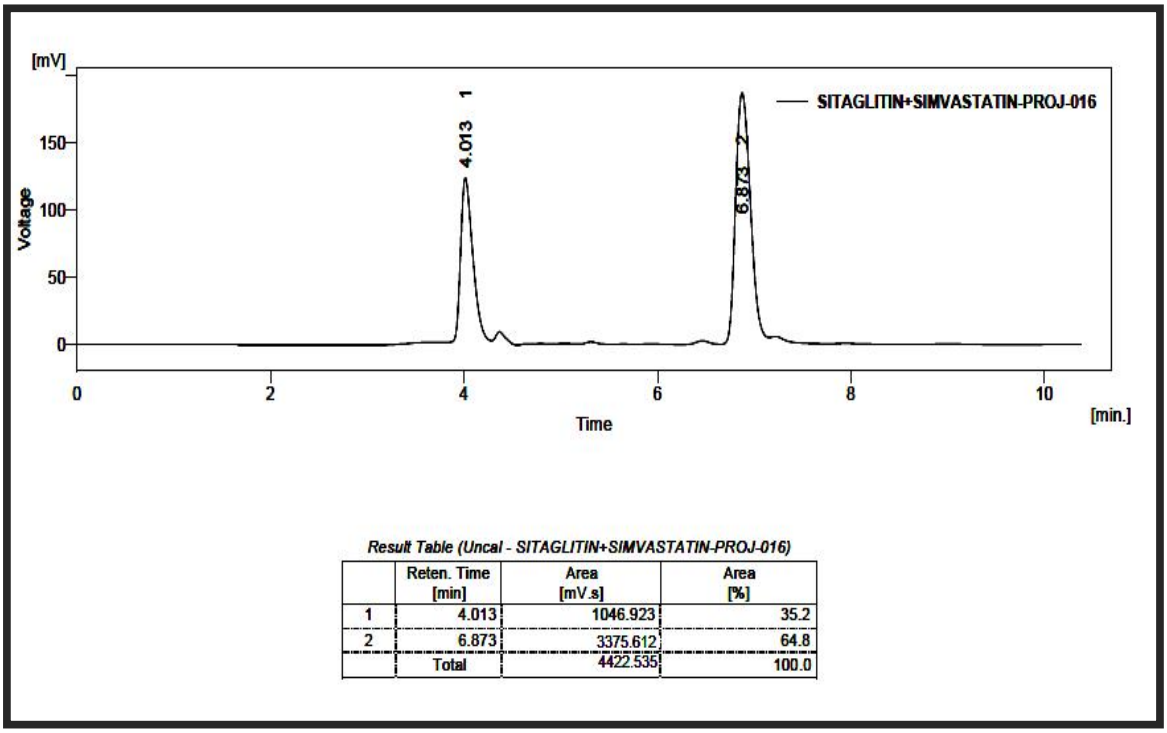
FIG – 59 CHROMATOGRAM FOR 80% RECOVERY FORMULATION



Result Table (Uncal - SITAGLITIN+SIMVASTATIN-PROJ-015)

	Reten. Time [min]	Area [mV.s]	Area [%]
1	4.013	959.609	33.4
2	6.867	3037.568	66.6
	Total	3997.177	100.0

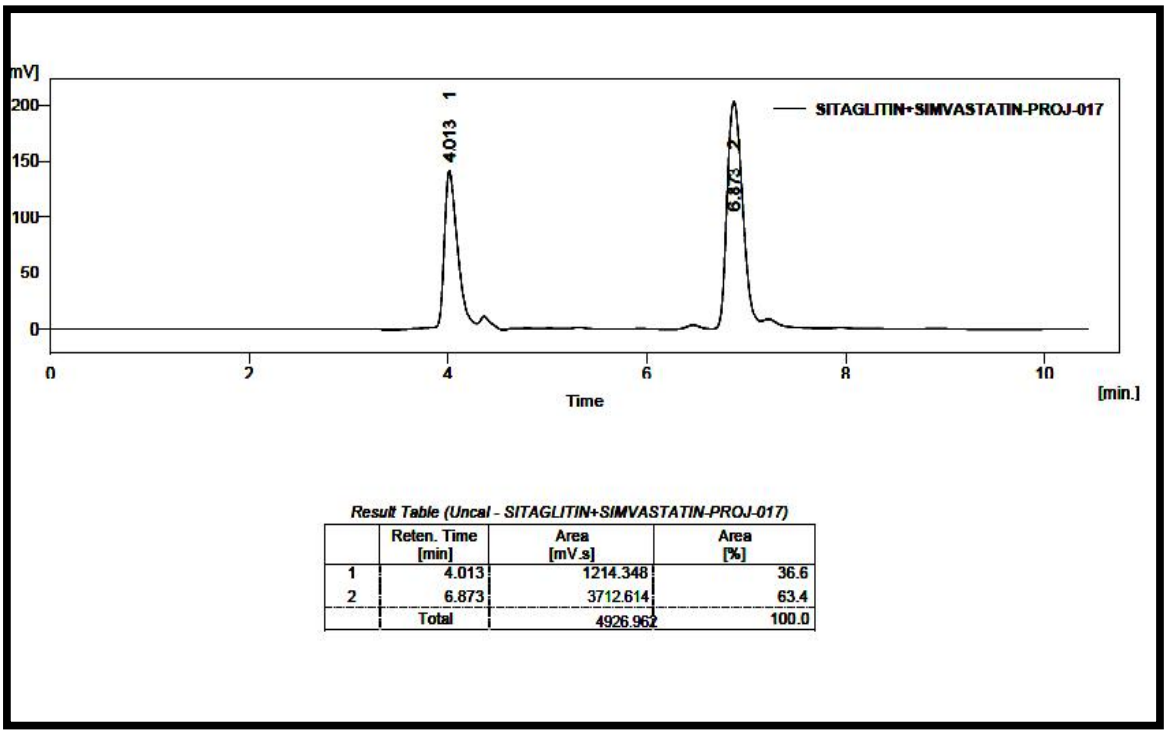
FIG – 60 CHROMATOGRAM FOR 100% RECOVERY FORMULATION



Result Table (Uncal - SITAGLITIN+SIMVASTATIN-PROJ-016)

	Reten. Time [min]	Area [mV.s]	Area [%]
1	4.013	1046.923	35.2
2	6.873	3375.612	64.8
	Total	4422.535	100.0

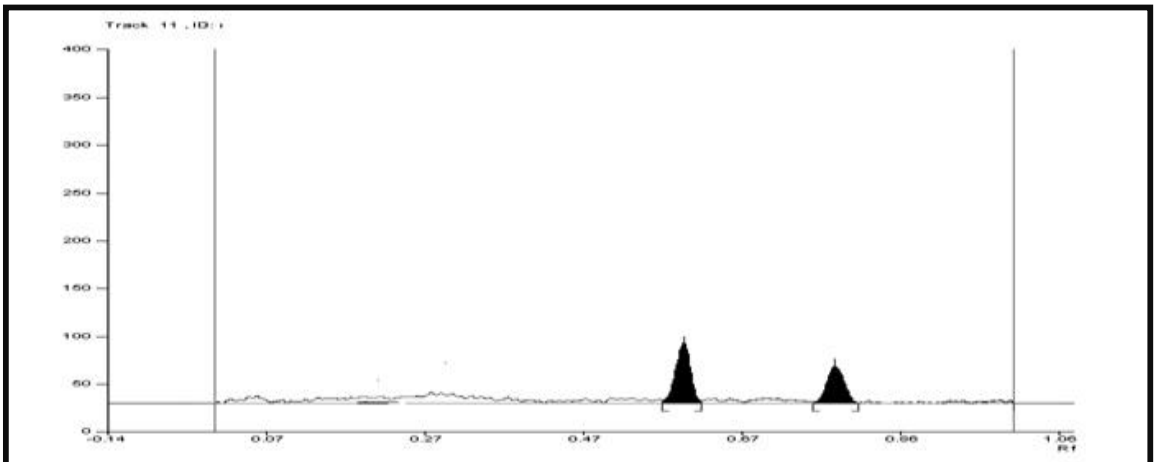
FIG – 61 CHROMATOGRAM FOR 120% RECOVERY FORMULATION



Result Table (Uncal - SITAGLITIN+SIMVASTATIN-PROJ-017)

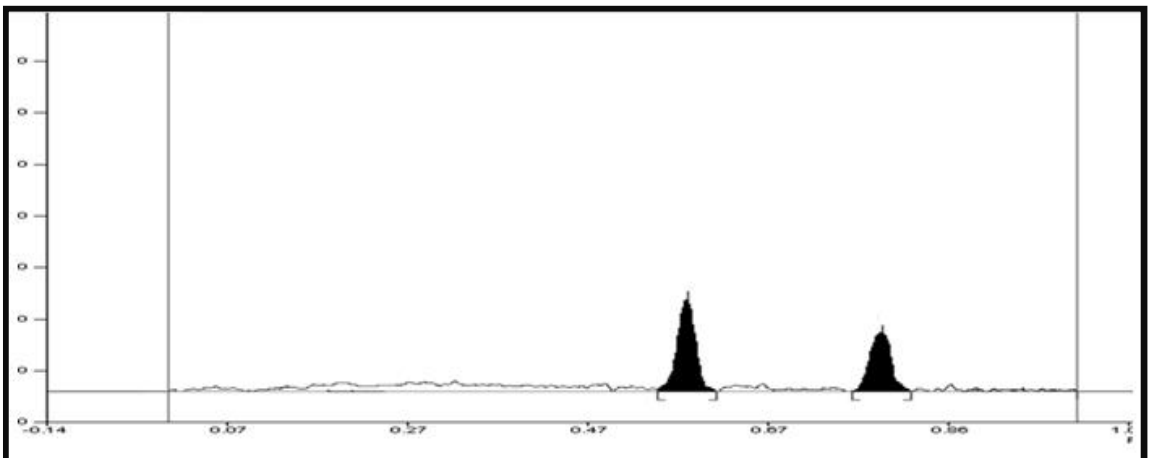
	Reten. Time [min]	Area [mV.s]	Area [%]
1	4.013	1214.348	36.6
2	6.873	3712.614	63.4
	Total	4926.962	100.0

FIG – 62 HPTLC LINEARITY CHROMATOGRAM OF SITAGLIPTIN PHOSPHATE AND SIMVASTATIN (40 ng/µl + 100 ng/µl)



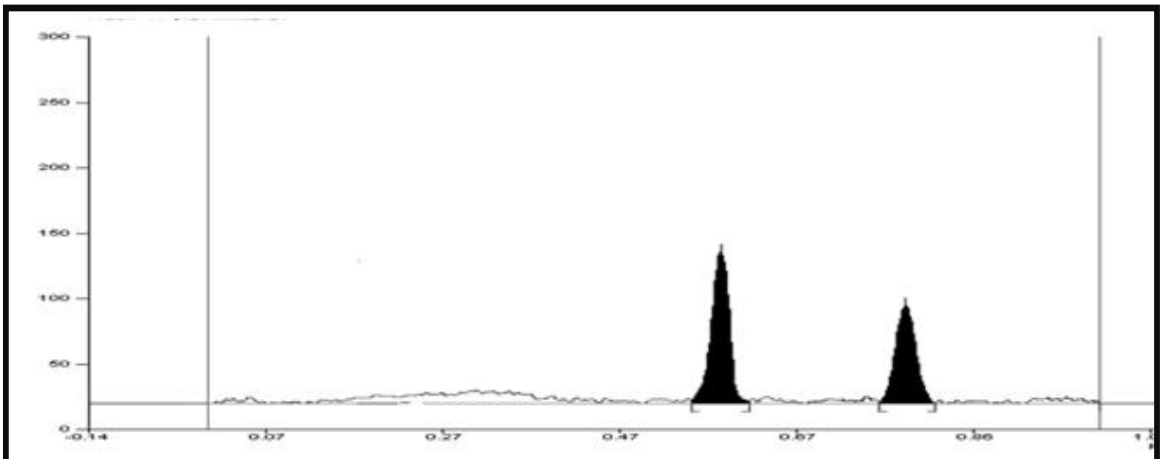
Peak	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %	Substance
1	0.56	2.6	0.59	62.1	44.01	0.61	1.9	1123.3	37.61	SIM
2.	0.76	1.4	0.78	36.8	37.36	0.79	0.9	954.21	34.38	SITA

FIG – 63 HPTLC LINEARITY CHROMATOGRAM OF SITAGLIPTIN PHOSPHATE AND SIMVASTATIN (80 ng/µl + 200 ng/µl)



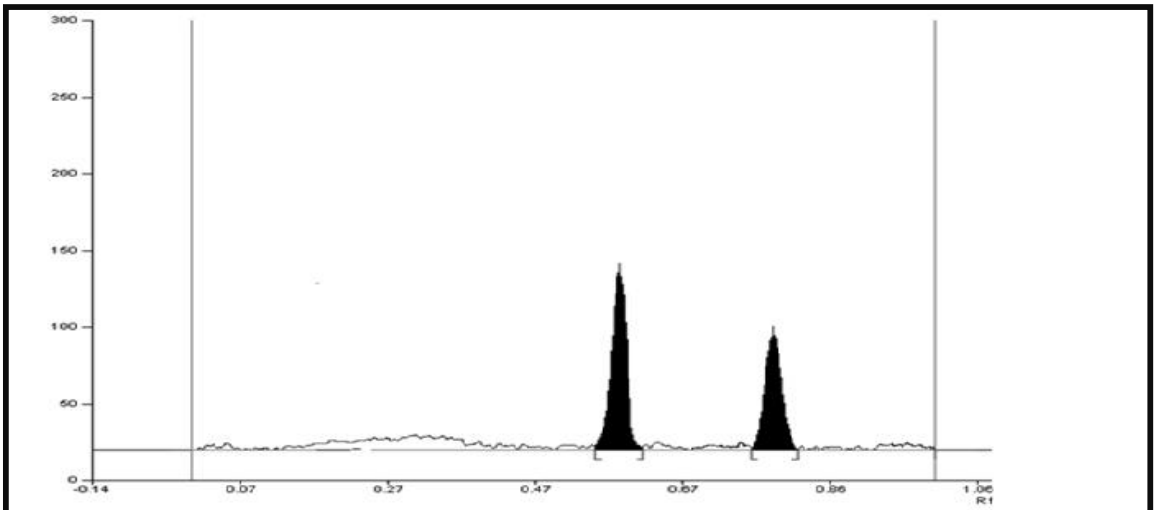
Peak	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %	Substance
1.	0.56	2.6	0.59	82.1	44.01	0.61	1.9	2246.62	36.64	SIM
2.	0.76	1.4	0.78	46.9	37.36	0.79	0.9	1908.95	33.42	SITA

FI G – 64 HPTLC LINEARITY CHROMATOGRAM OF SITAGLIPTIN PHOSPHATE AND SIMVASTATIN (120 ng/µl+ 300 ng/µl)



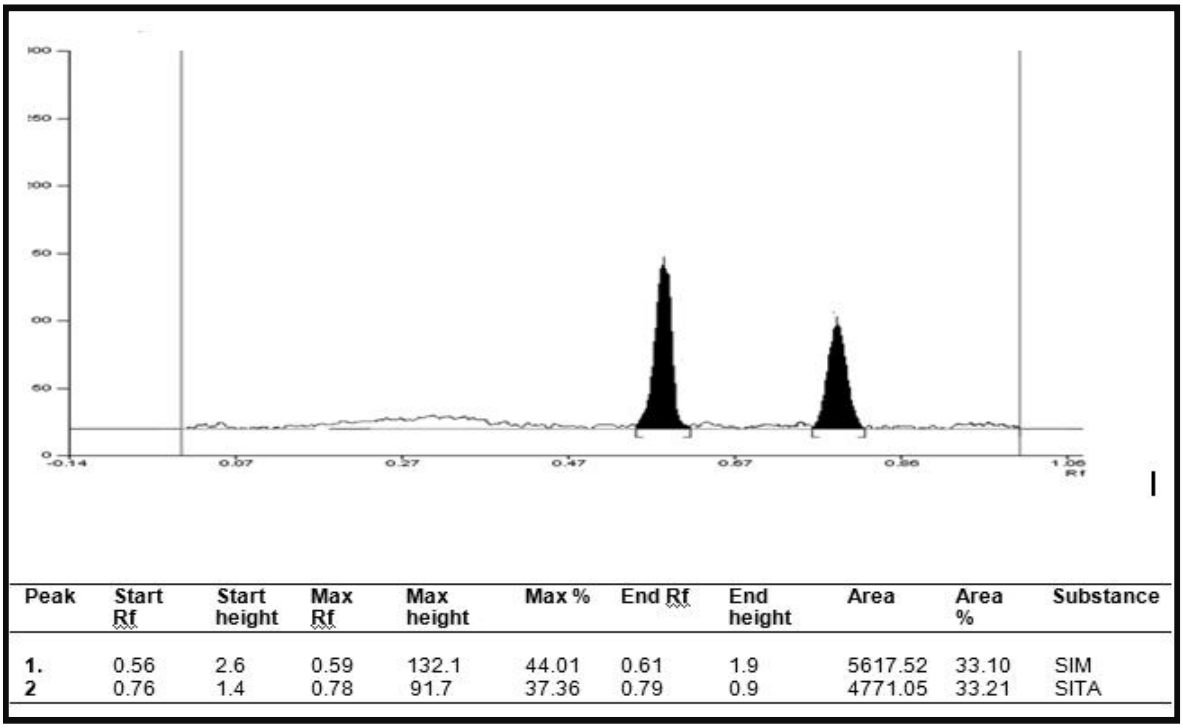
Peak	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %	Substance
1	0.56	2.6	0.59	102.1	44.01	0.62	1.9	3298.65	36.31	SIM
2	0.76	1.4	0.78	67.5	37.36	0.81	0.9	2862.23	34.12	SITA

FIG – 65 HPTLC LINEARITY CHROMATOGRAM OF SITAGLIPTIN PHOSPHATE AND SIMVASTATIN (160 ng/µl +400 ng/µl)



Peak	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %	Substance
1	0.56	2.6	0.59	129.1	44.01	0.63	1.9	4378.30	33.76	SIM
2.	0.76	1.4	0.79	88.9	37.36	0.81	0.9	3816.84	32.43	SITA

FIG – 66 HPTLC LINEARITY CHROMATOGRAM OF SITAGLIPTIN PHOSPHATE AND SIMVASTATIN (100 ng/µl+ 500 ng/µl)



Peak	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %	Substance
1.	0.56	2.6	0.59	132.1	44.01	0.61	1.9	5617.52	33.10	SIM
2	0.76	1.4	0.78	91.7	37.36	0.79	0.9	4771.05	33.21	SITA

FIG - 67 CALIBRATION CURVE OF SITAGLIPTIN PHOSPHATE BY HPTLC METHOD

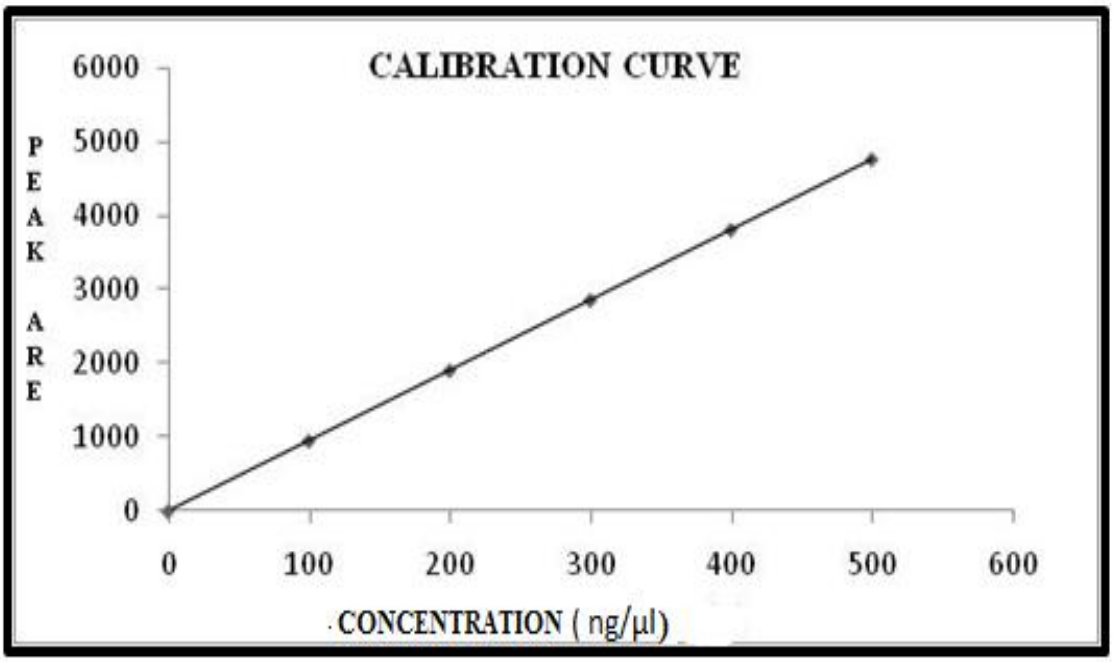


FIG 68 - CALIBRATION CURVE OF SIMVASTATIN BY HPTLC METHOD

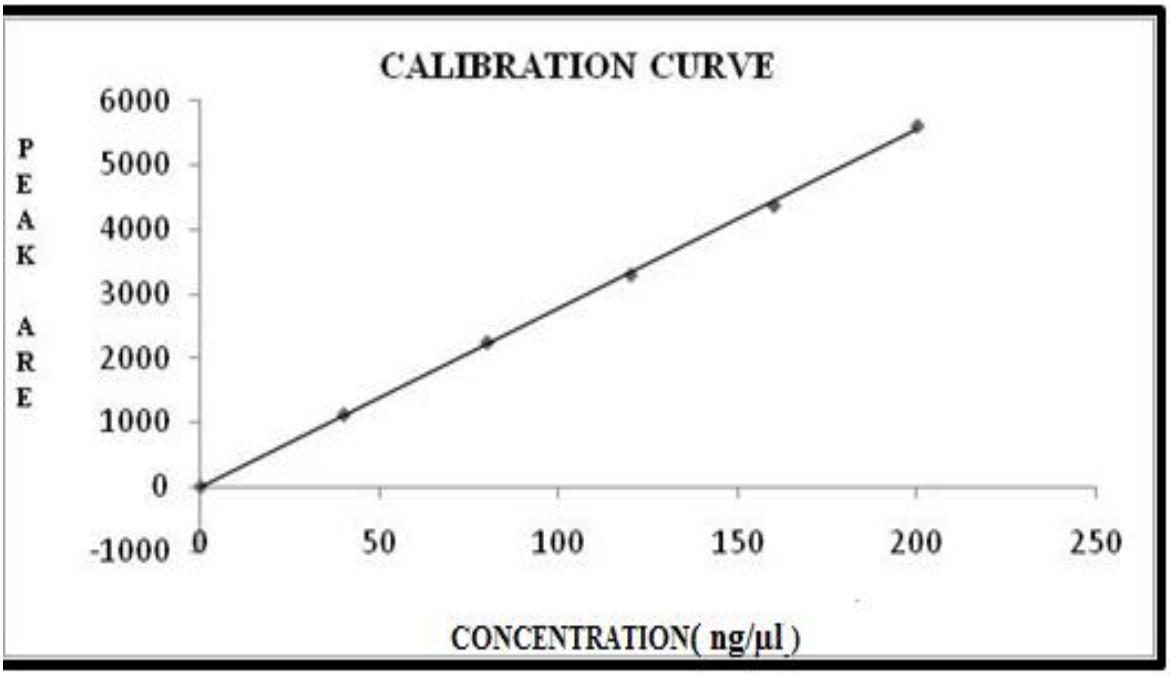
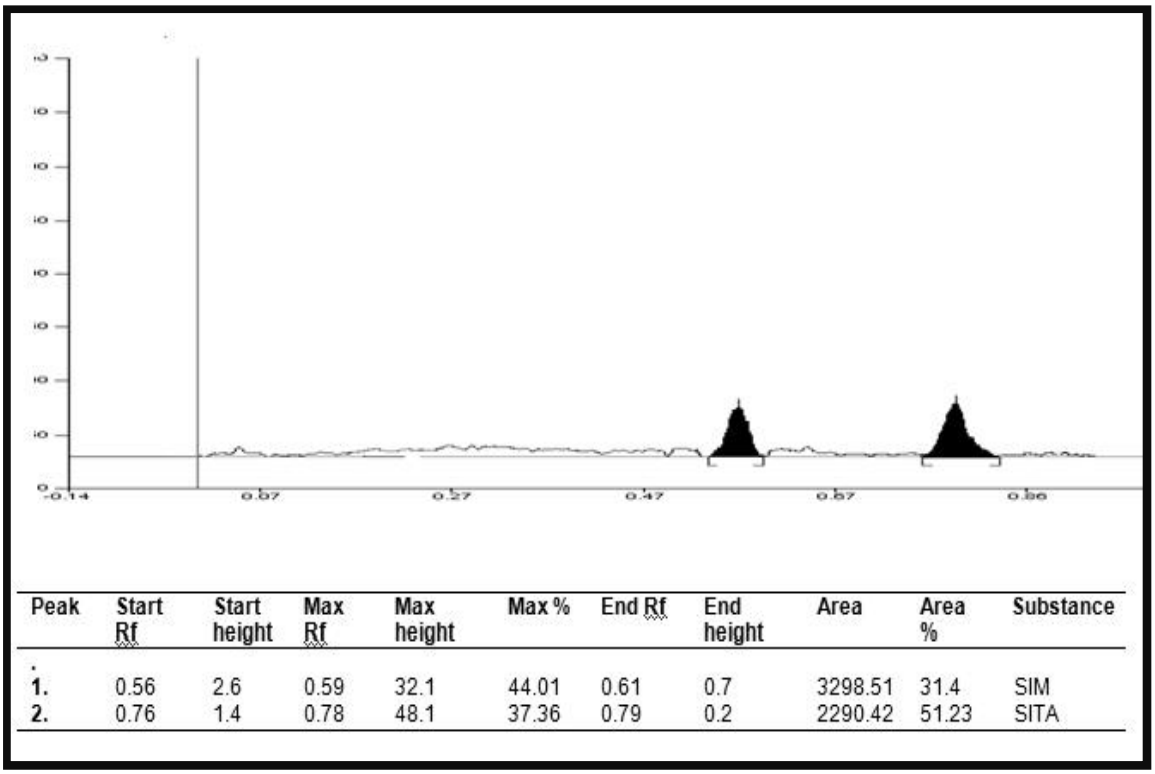
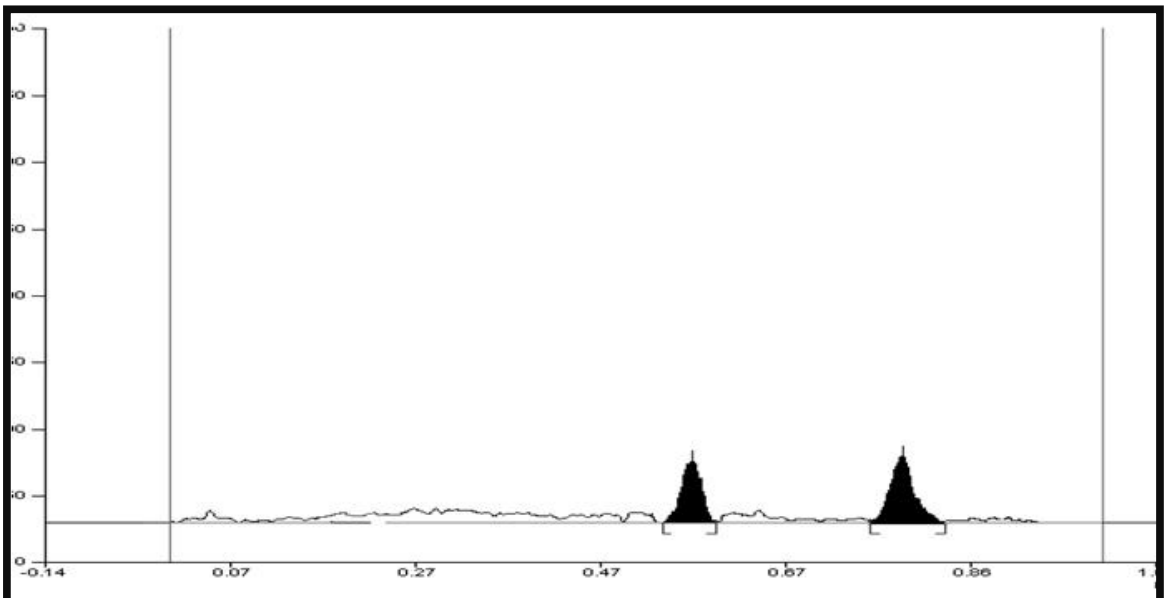


FIG – 69 CHROMATOGRAM FOR FORMULATION – 1



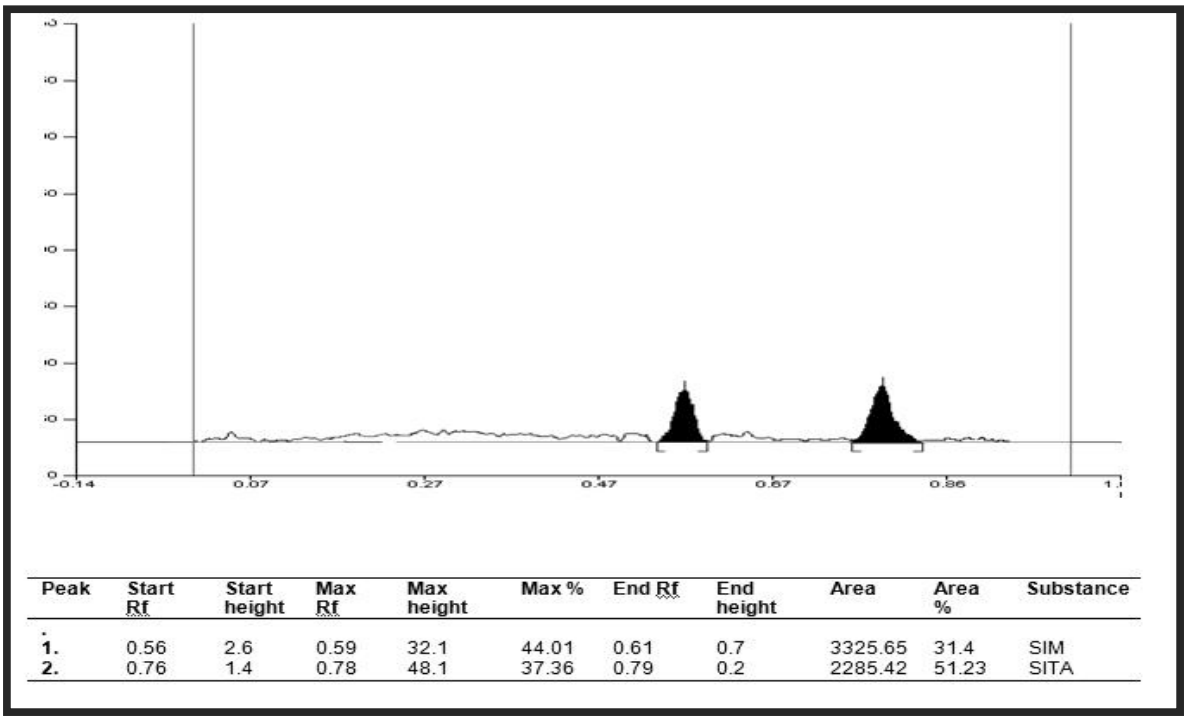
Peak	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %	Substance
1.	0.56	2.6	0.59	32.1	44.01	0.61	0.7	3298.51	31.4	SIM
2.	0.76	1.4	0.78	48.1	37.36	0.79	0.2	2290.42	51.23	SITA

FIG – 70 CHROMATOGRAM FOR FORMULATION -2



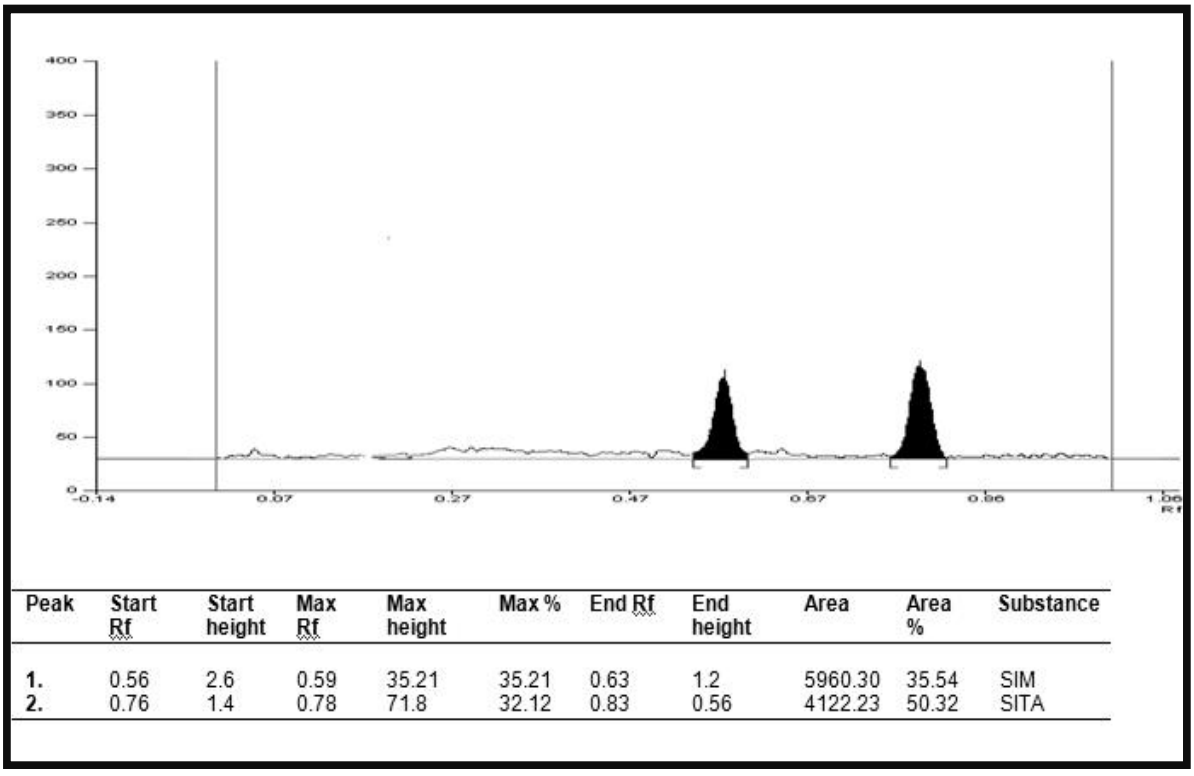
Peak	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %	Substance
1.	0.56	2.6	0.59	32.1	44.01	0.61	0.7	3310.89	31.4	SIM
2.	0.76	1.4	0.78	48.1	37.36	0.79	0.2	2279.35	51.23	SITA

FIG – 71 CHROMATOGRAM FOR FORMULATION – 3



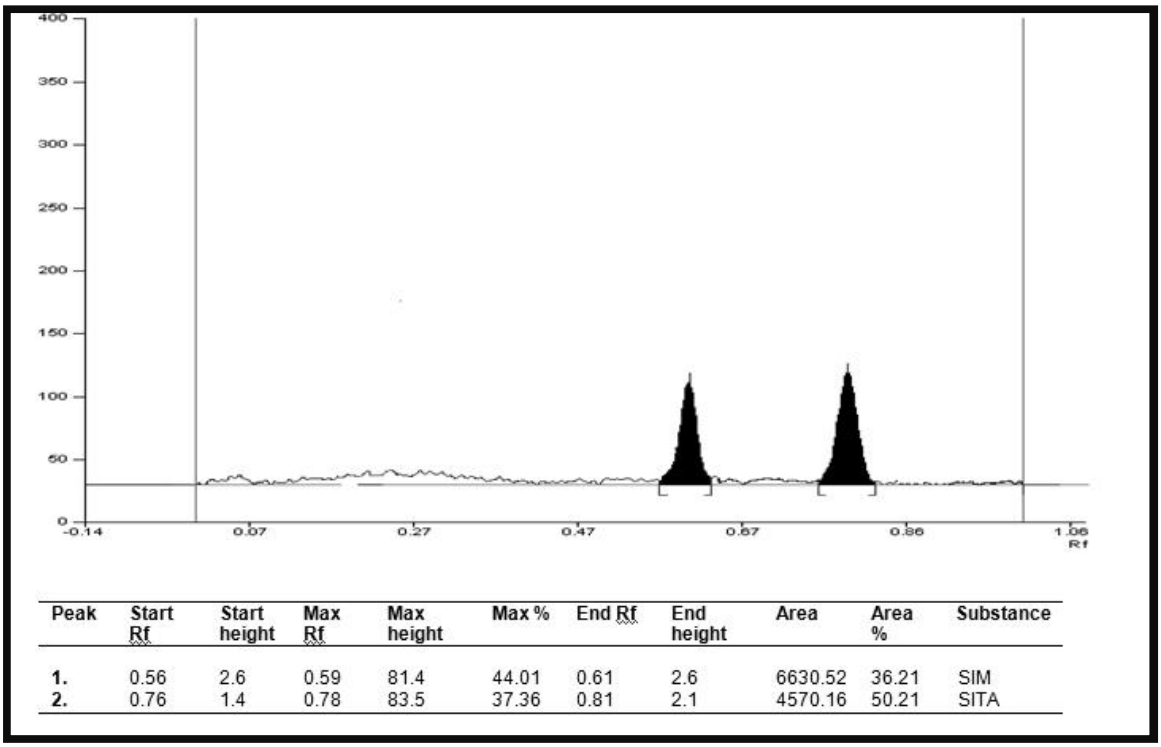
Peak	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %	Substance
1.	0.56	2.6	0.59	32.1	44.01	0.61	0.7	3325.65	31.4	SIM
2.	0.76	1.4	0.78	48.1	37.36	0.79	0.2	2285.42	51.23	SITA

FIG – 72 CHROMATOGRAM FOR 80% RECOVERY ANALYSIS



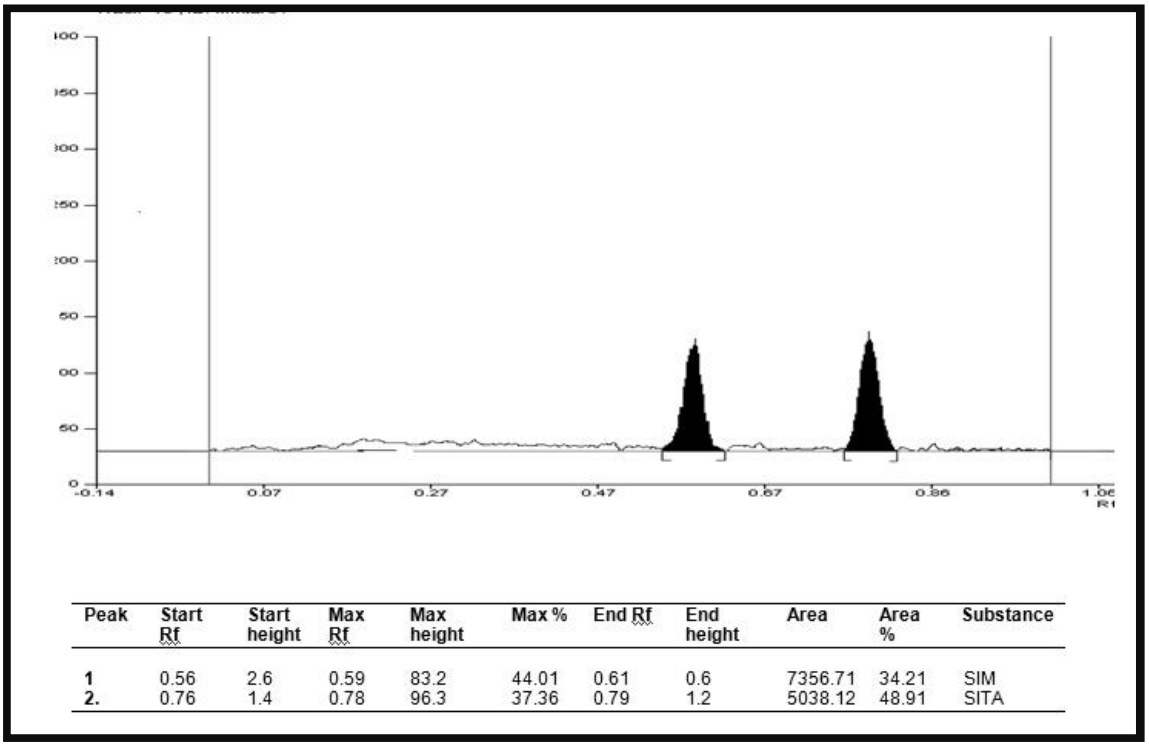
Peak	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %	Substance
1.	0.56	2.6	0.59	35.21	35.21	0.63	1.2	5960.30	35.54	SIM
2.	0.76	1.4	0.78	71.8	32.12	0.83	0.56	4122.23	50.32	SITA

FIG – 73 CHROMATOGRAM FOR 100% RECOVERY ANALYSIS



Peak	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %	Substance
1.	0.56	2.6	0.59	81.4	44.01	0.61	2.6	6630.52	36.21	SIM
2.	0.76	1.4	0.78	83.5	37.36	0.81	2.1	4570.16	50.21	SITA

FIG – 74 CHROMATOGRAM FOR 120% RECOVERY ANALYSIS



Peak	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %	Substance
1	0.56	2.6	0.59	83.2	44.01	0.61	0.6	7356.71	34.21	SIM
2.	0.76	1.4	0.78	96.3	37.36	0.79	1.2	5038.12	48.91	SITA

FIG - 75 IR SPECTRA OF THIOCOLCHICOSIDE

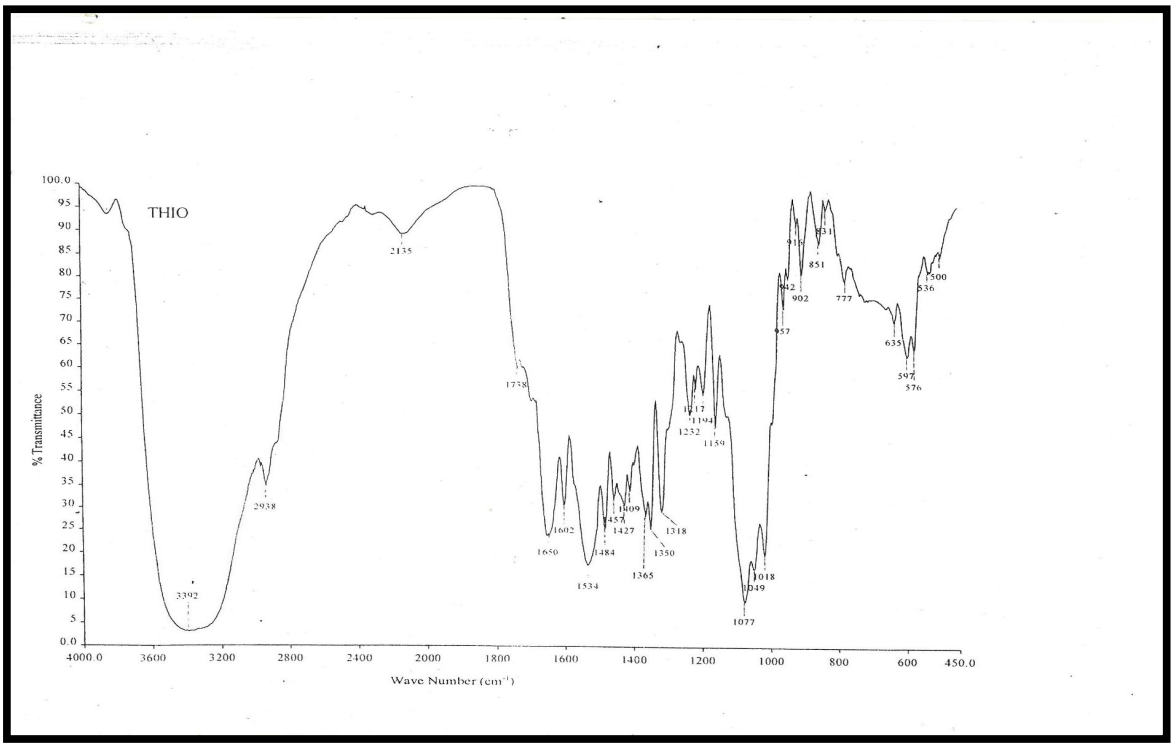


FIG -76 IR SPECTRA OF KETOPROFEN

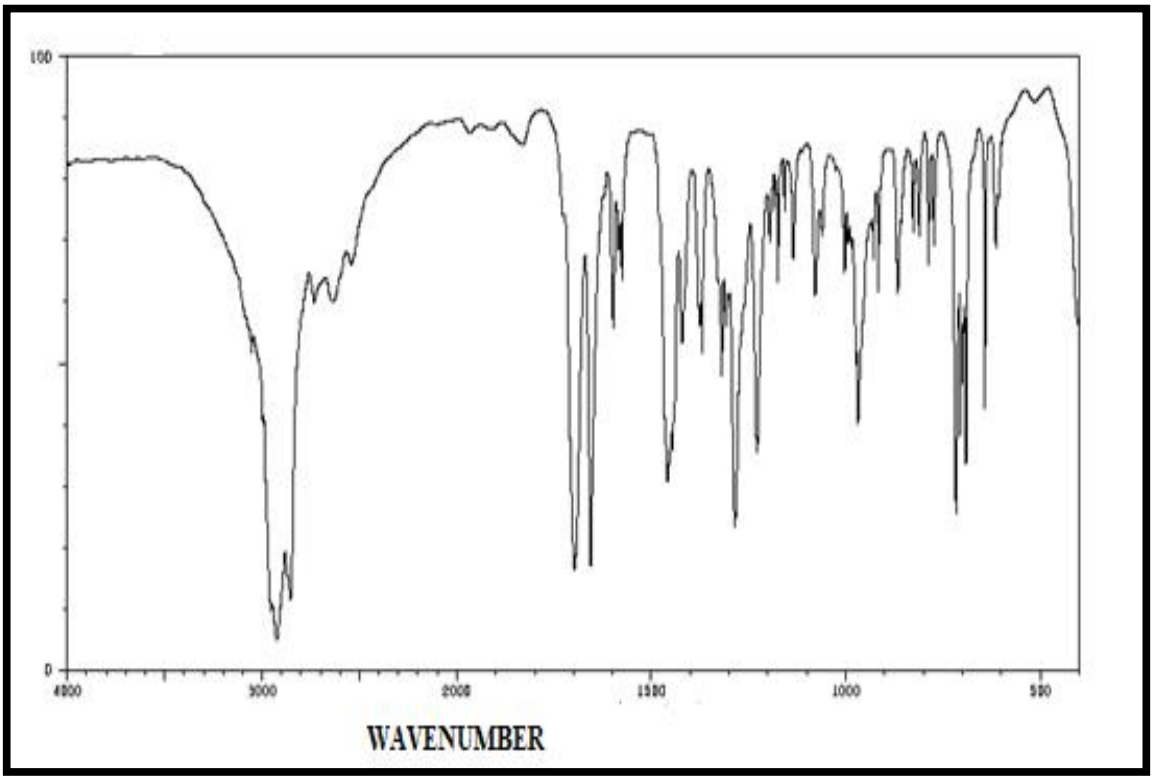


FIG - 77 OVERLAIN SPECTRA OF THIOCOLCHICOSIDE AND KETOPROFEN

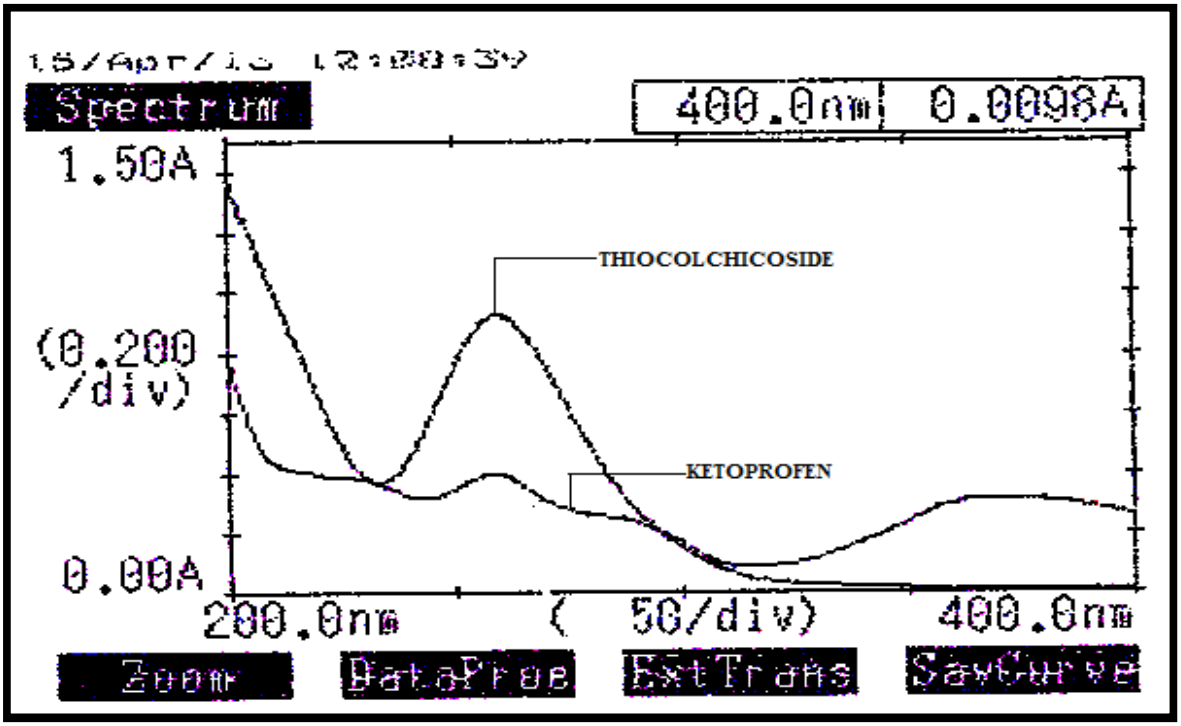


FIG – 78 OVERLAIN FIRST ORDER DERIVATIVE SPECTRAOF THIOCOLCHICOSIDE AND KETOPROFEN

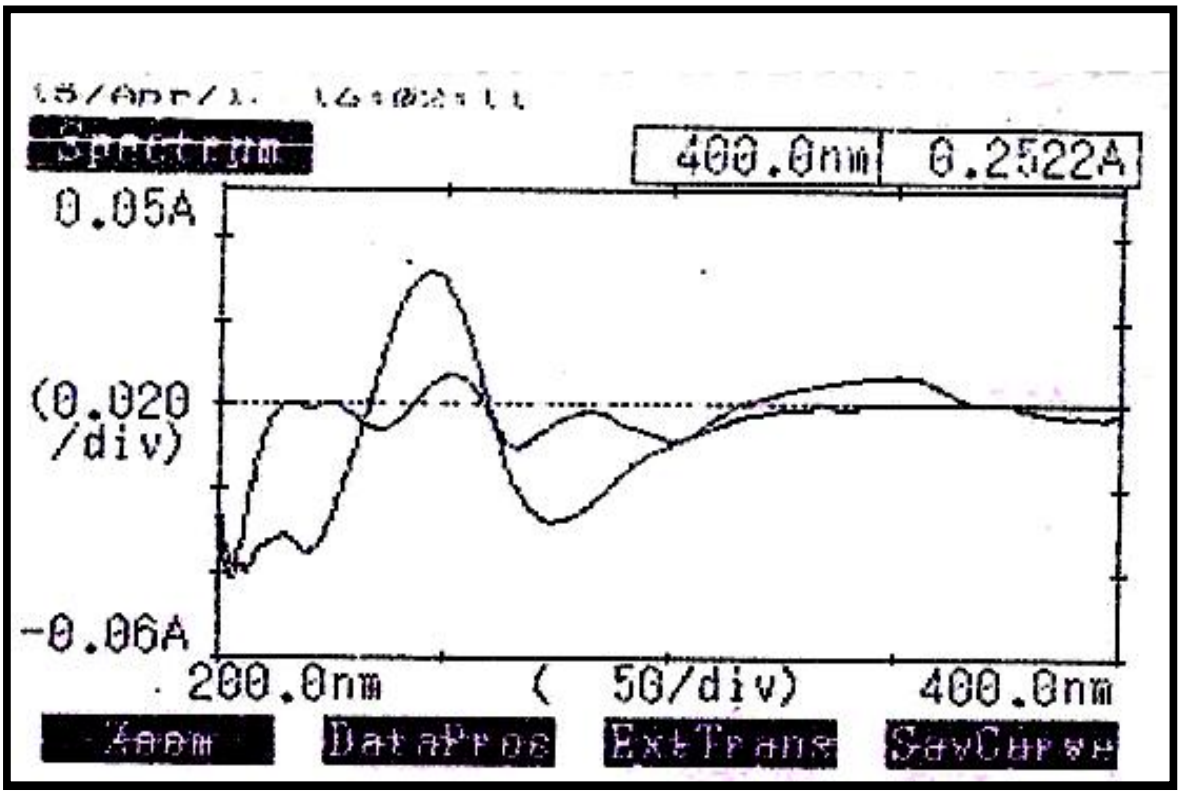


FIG -79 CALIBRATION CURVE OF THIOCOLCHICOSIDE

(FIRST ORDER DERIVATIVE SPECTRA)

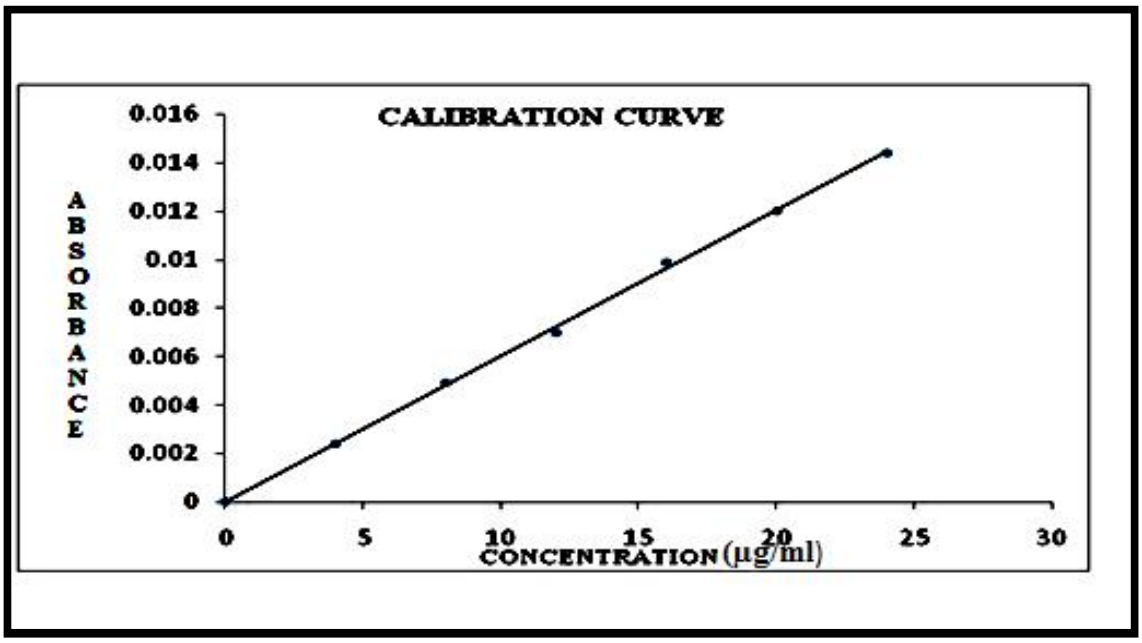


FIG - 80 CALIBRATION CURVE OF KETOPROFEN (FIRST ORDER DERIVATIVE SPECTRA)

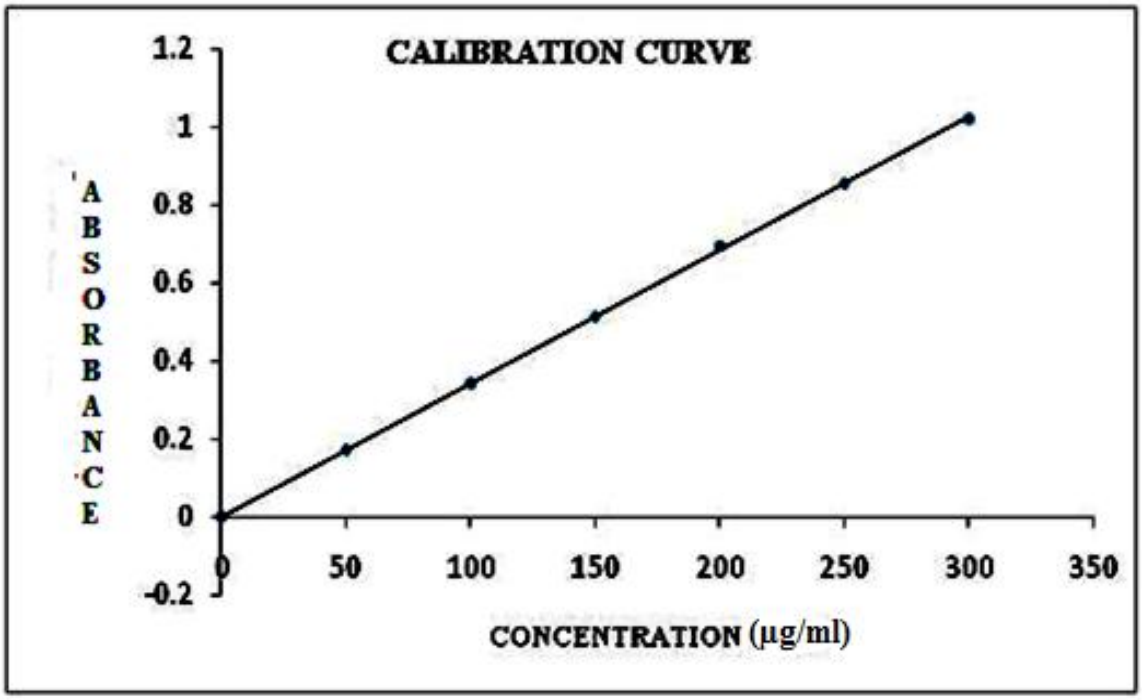
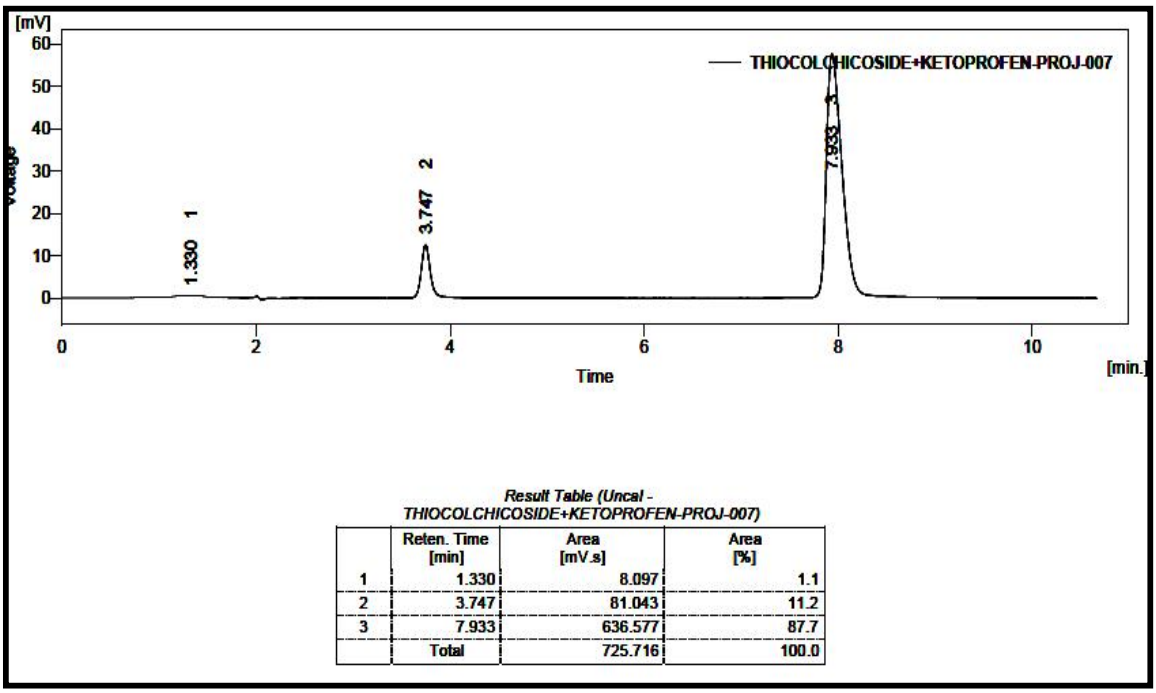


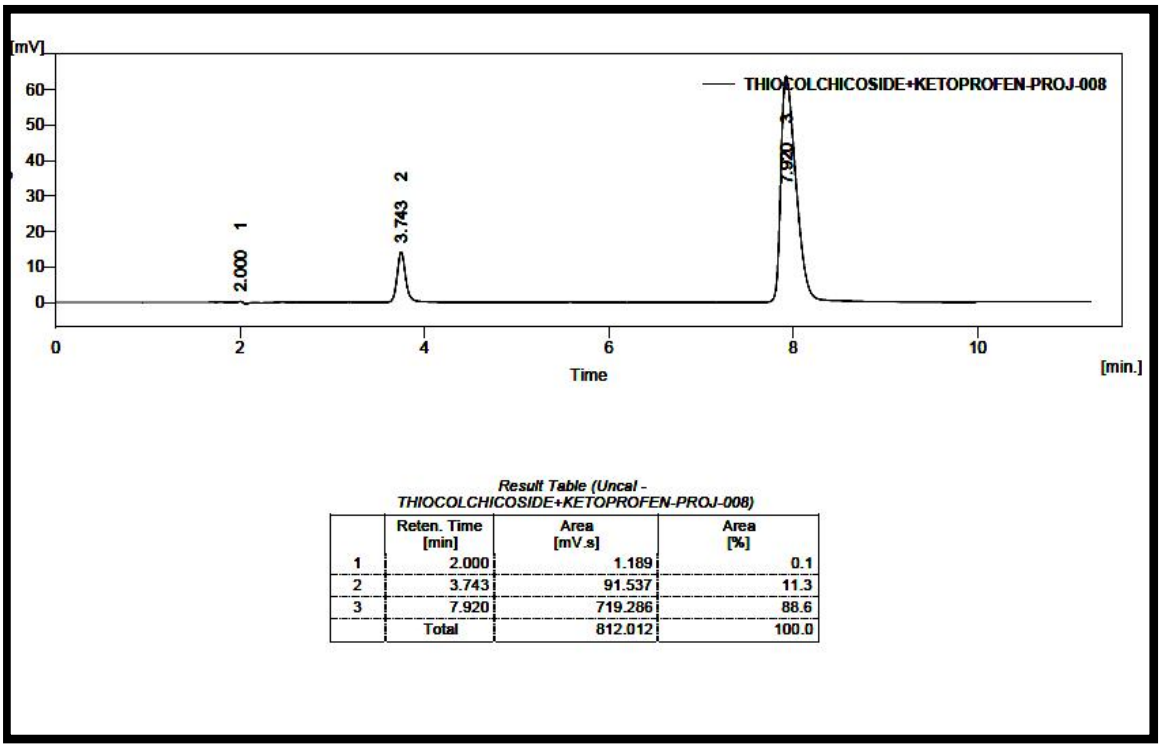
FIG – 81 RP-HPLC LINEARITY CHROMATOGRAM OF KETOPROFEN AND THIOCOLCHICOSIDE (80 µg/ml + 6.4 µg/ml)



Result Table (Uncal - THIOCOLCHICOSIDE+KETOPROFEN-PROJ-007)

	Reten. Time [min]	Area [mV.s]	Area [%]
1	1.330	8.097	1.1
2	3.747	81.043	11.2
3	7.933	636.577	87.7
	Total	725.716	100.0

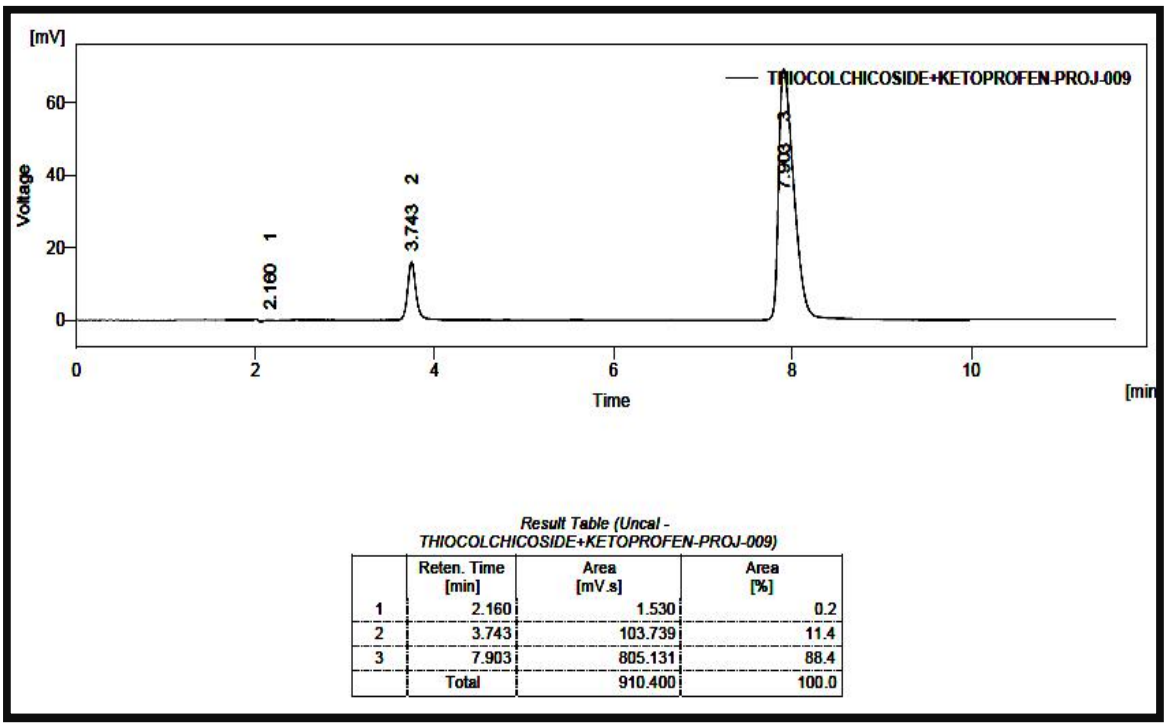
FIG -- 82 RP - HPLC LINEARITY CHROMATOGRAM OF KETOPROFEN AND THIOCOLCHICOSIDE (90 µg/ml + 7.2 µg/ml)



Result Table (Uncal - THIOCOLCHICOSIDE+KETOPROFEN-PROJ-008)

	Reten. Time [min]	Area [mV.s]	Area [%]
1	2.000	1.189	0.1
2	3.743	91.537	11.3
3	7.920	719.286	88.6
	Total	812.012	100.0

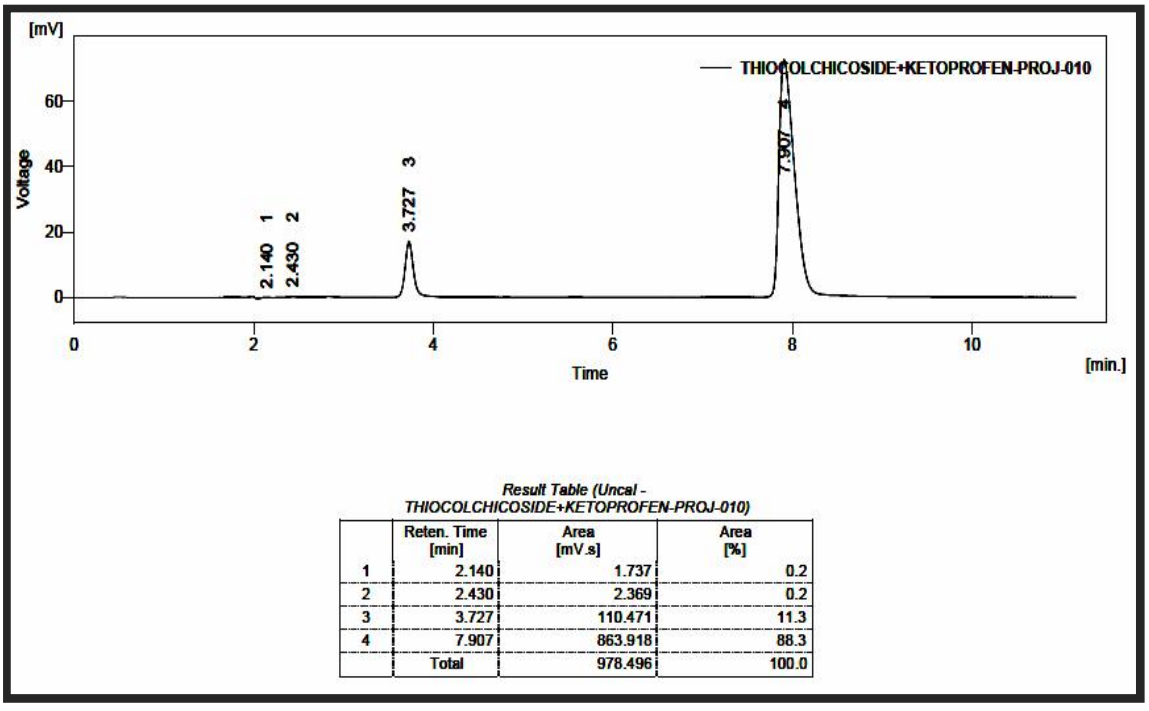
FIG – 83 RP-HPLC LINEARITY CHROMATOGRAM OF KETOPROFEN AND THIOCOLCHICOSIDE (100 µg/ml + 8 µg/ml)



Result Table (Uncal - THIOCOLCHICOSIDE+KETOPROFEN-PROJ-009)

	Reten. Time [min]	Area [mV.s]	Area [%]
1	2.160	1.530	0.2
2	3.743	103.739	11.4
3	7.903	805.131	88.4
	Total	910.400	100.0

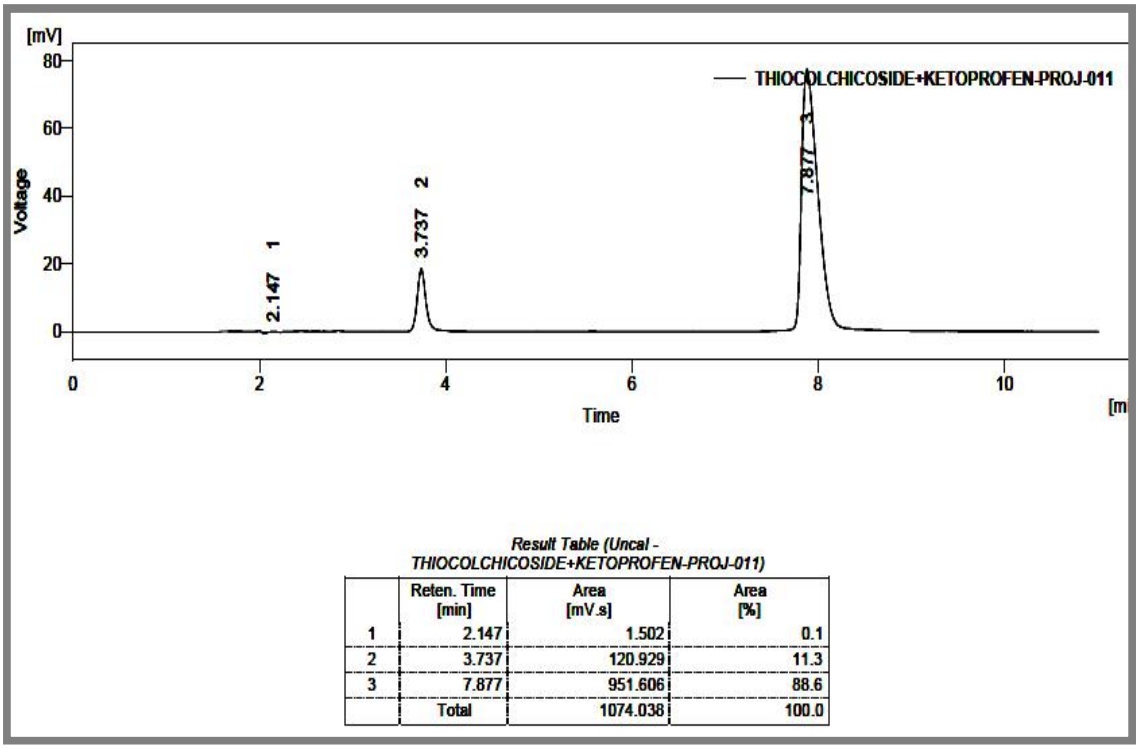
FIG – 84 RP-HPLC LINEARITY CHROMATOGRAM OF KETOPROFEN AND THIOCOLCHICOSIDE (110 µg/ml + 8.8 µg/ml)



Result Table (Uncal - THIOCOLCHICOSIDE+KETOPROFEN-PROJ-010)

	Reten. Time [min]	Area [mV.s]	Area [%]
1	2.140	1.737	0.2
2	2.430	2.369	0.2
3	3.727	110.471	11.3
4	7.907	863.918	88.3
	Total	978.496	100.0

FIG – 85 RP-HPLC LINEARITY CHROMATOGRAM OF KETOPROFEN AND THIOCOLCHICOSIDE (120 µg/ml + 9.6 µg/ml)



Result Table (Uncal - THIOCOLCHICOSIDE+KETOPROFEN-PROJ-011)

	Reten. Time [min]	Area [mV.s]	Area [%]
1	2.147	1.502	0.1
2	3.737	120.929	11.3
3	7.877	951.606	88.6
	Total	1074.038	100.0

FIG – 86 CALIBRATION CURVE OF KETOPROFEN BY RP-HPLC METHOD

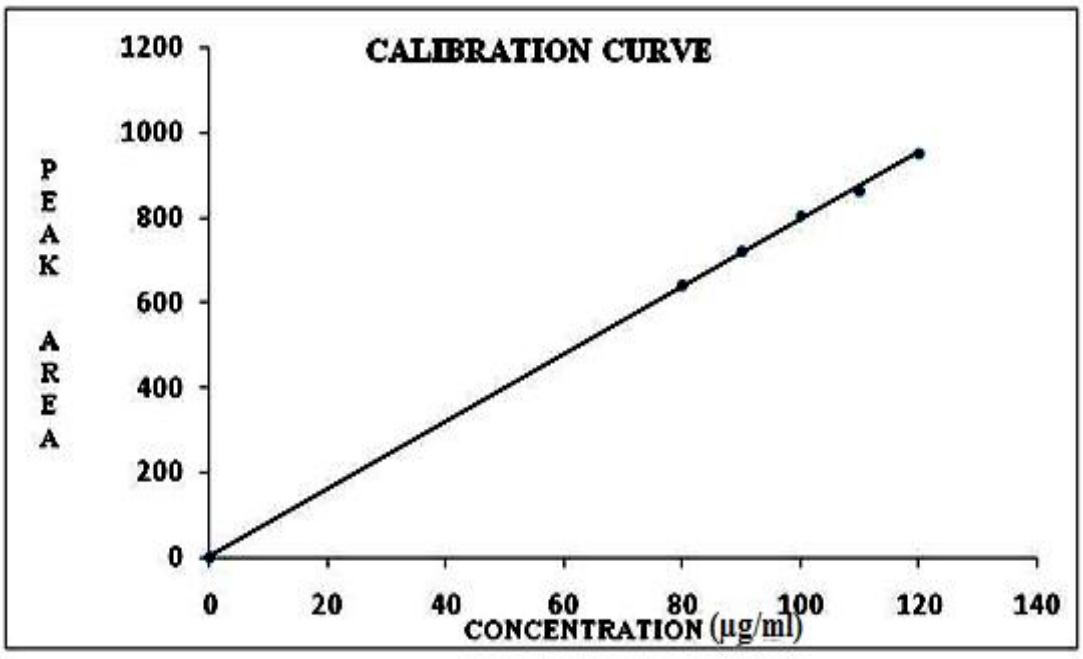


FIG – 87 CALIBRATION CURVE OF THIOCOLCHICOSIDE BY RP-HPLC METHOD

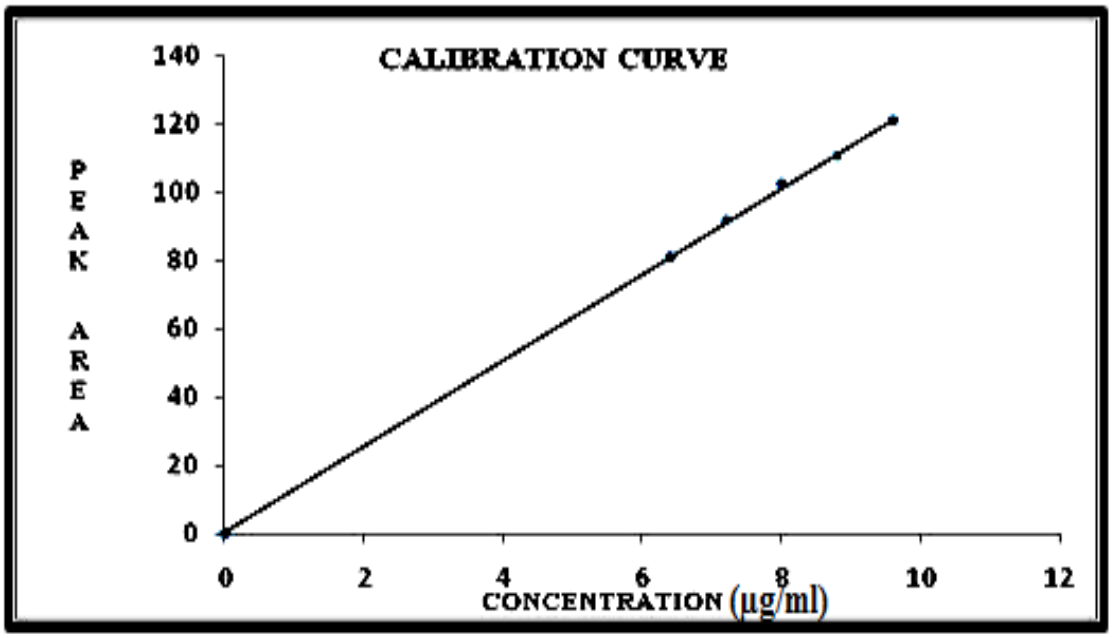
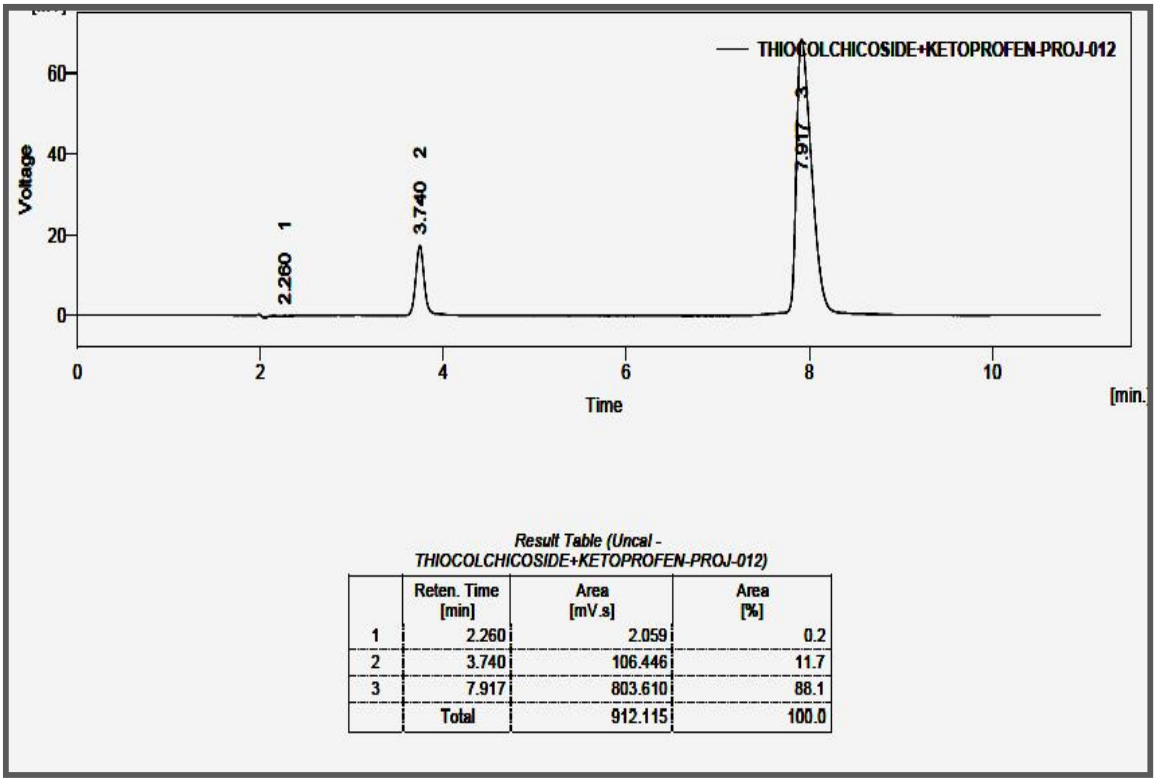


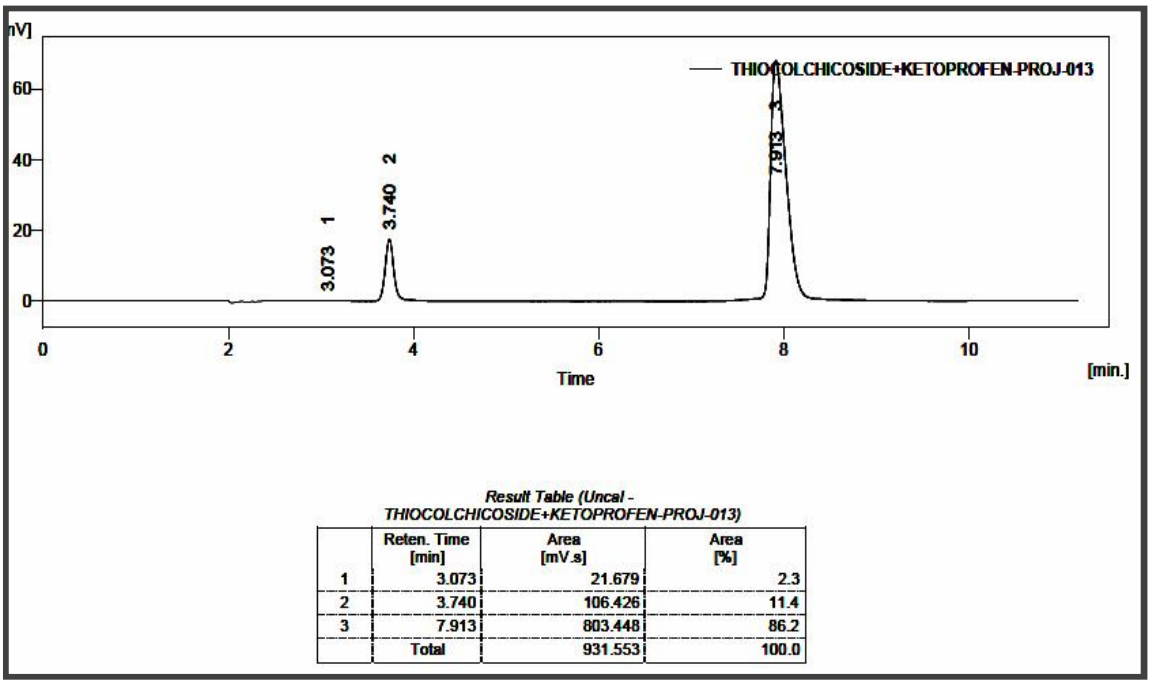
FIG – 88 CHROMATOGRAM FOR FORMULATION – 1



Result Table (Uncal - THIOCOLCHICOSIDE+KETOPROFEN-PROJ-012)

	Reten. Time [min]	Area [mV.s]	Area [%]
1	2.260	2.059	0.2
2	3.740	106.446	11.7
3	7.917	803.610	88.1
	Total	912.115	100.0

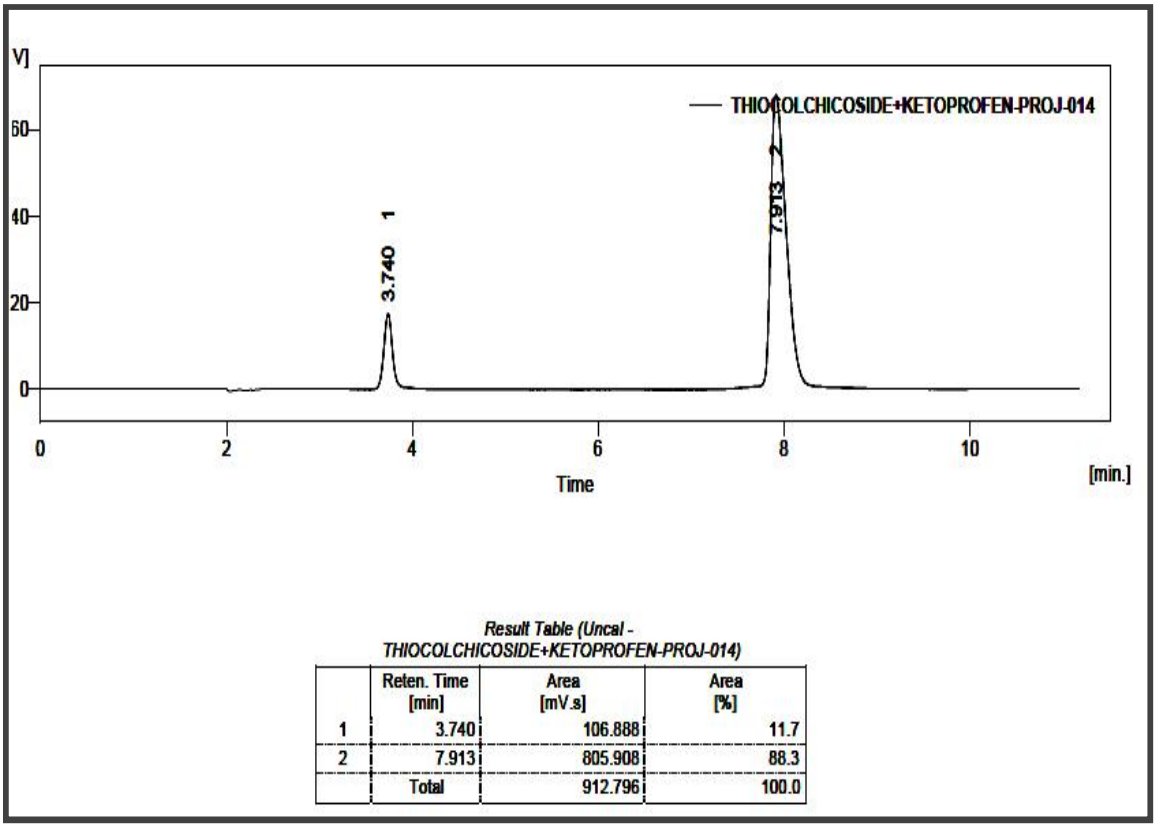
FIG – 89 CHROMATOGRAM FOR FORMULATION – 2



Result Table (Uncal - THIOCOLCHICOSIDE+KETOPROFEN-PROJ-013)

	Reten. Time [min]	Area [mV.s]	Area [%]
1	3.073	21.679	2.3
2	3.740	106.426	11.4
3	7.913	803.448	86.2
	Total	931.553	100.0

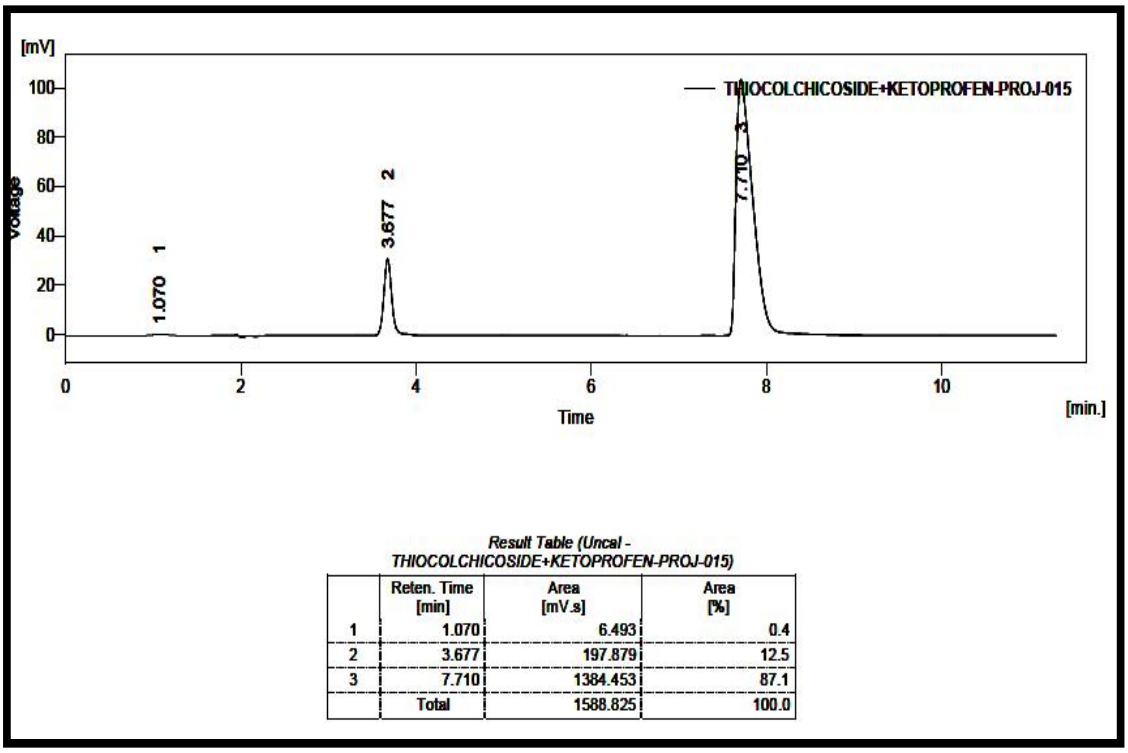
FIG – 90 CHROMATOGRAM FOR FORMULATION – 3



Result Table (Uncal - THIOCOLCHICOSIDE+KETOPROFEN-PROJ-014)

	Reten. Time [min]	Area [mV.s]	Area [%]
1	3.740	106.888	11.7
2	7.913	805.908	88.3
	Total	912.796	100.0

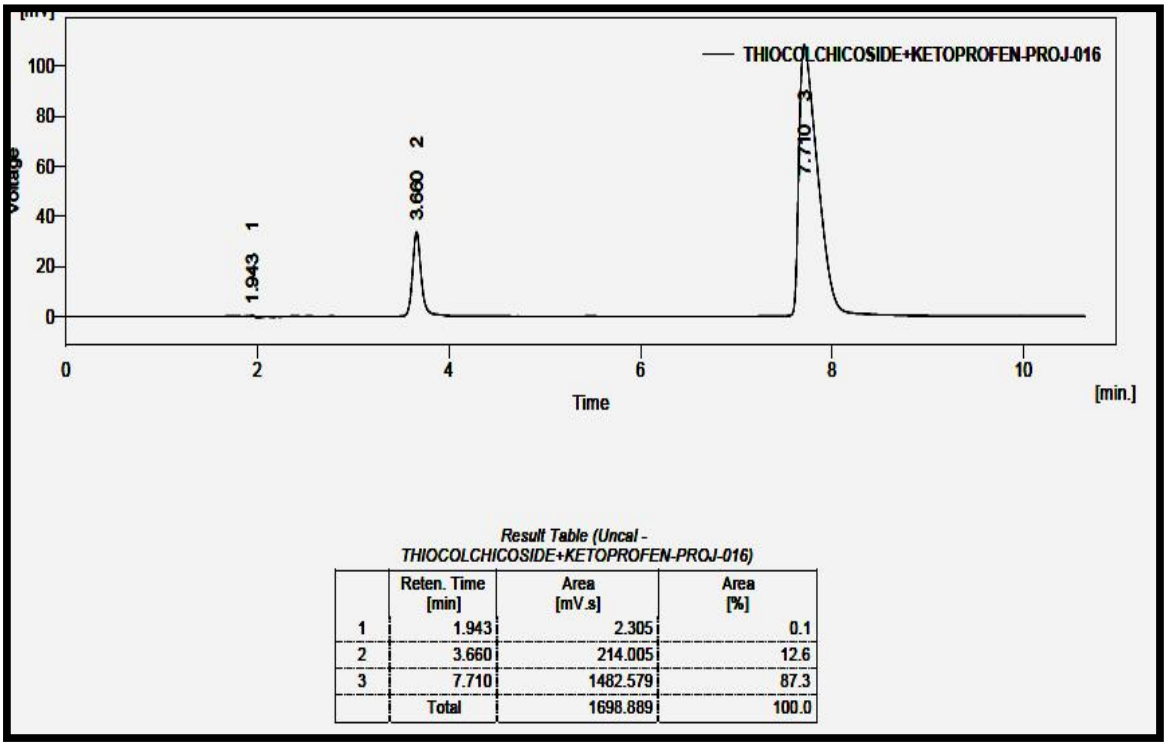
FIG – 91 CHROMATOGRAM FOR 80% RECOVERY FORMULATION



Result Table (Uncal - THIOCOLCHICOSIDE+KETOPROFEN-PROJ-015)

	Reten. Time [min]	Area [mV.s]	Area [%]
1	1.070	6.493	0.4
2	3.677	197.879	12.5
3	7.710	1384.453	87.1
	Total	1588.825	100.0

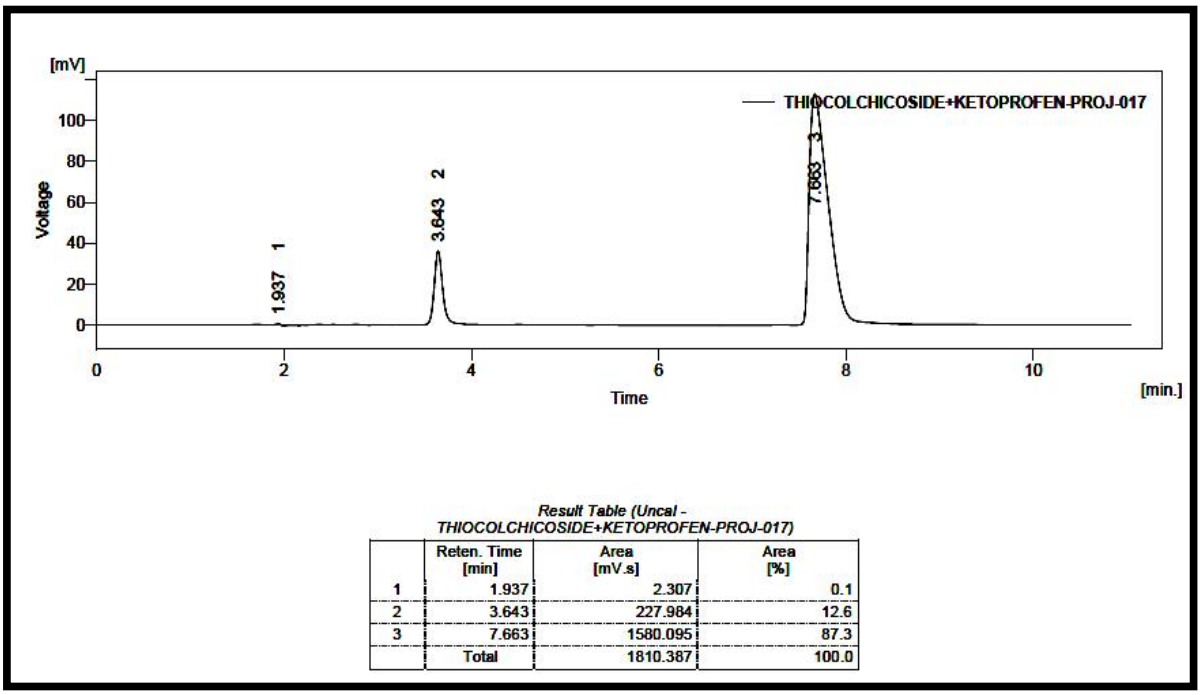
FIG – 92 CHROMATOGRAM FOR 100% RECOVERY FORMULATION



Result Table (Uncal - THIOCOLCHICOSIDE+KETOPROFEN-PROJ-016)

	Reten. Time [min]	Area [mV.s]	Area [%]
1	1.943	2.305	0.1
2	3.660	214.005	12.6
3	7.710	1482.579	87.3
	Total	1698.889	100.0

FIG – 93 CHROMATOGRAM FOR 120% RECOVERY FORMULATION



Result Table (Uncal - THIOCOLCHICOSIDE+KETOPROFEN-PROJ-017)

	Reten. Time [min]	Area [mV.s]	Area [%]
1	1.937	2.307	0.1
2	3.643	227.984	12.6
3	7.663	1580.095	87.3
	Total	1810.387	100.0

FIG 94 – IR SPECTRA OF DESLORATADINE

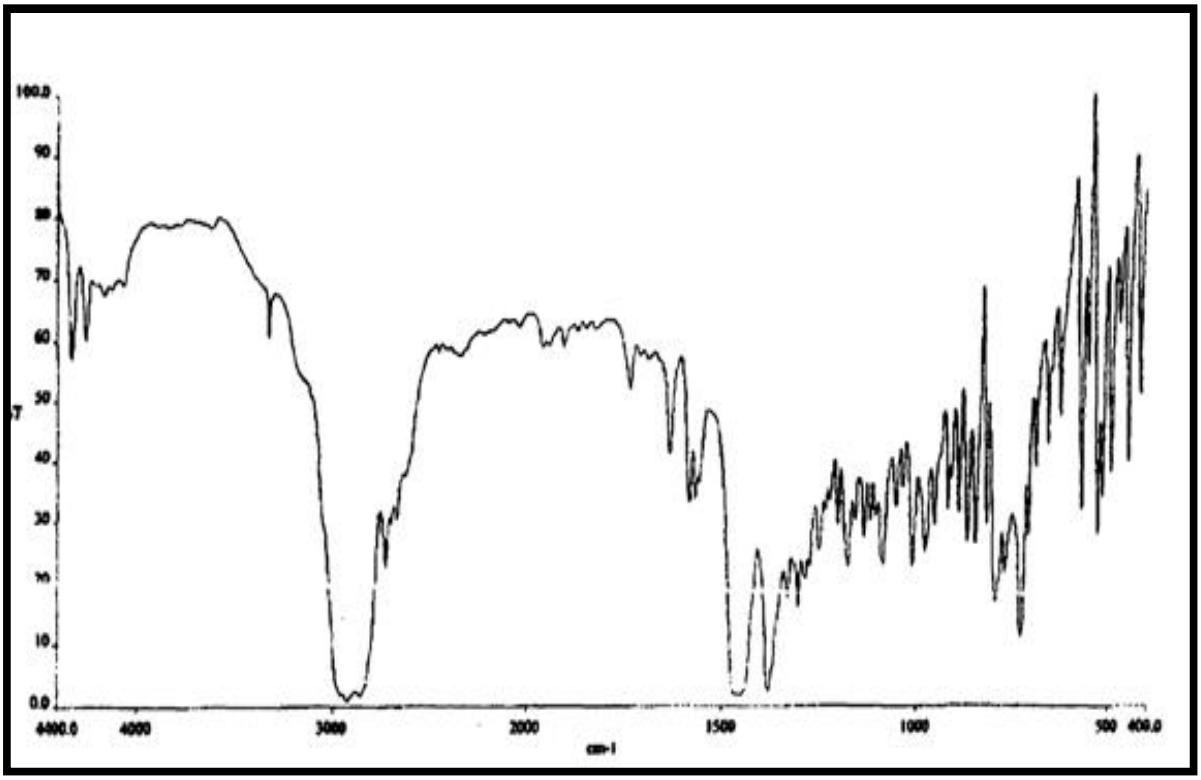


FIG 95– IR SPECTRA OF AMBROXOL HYDROCHLORIDE

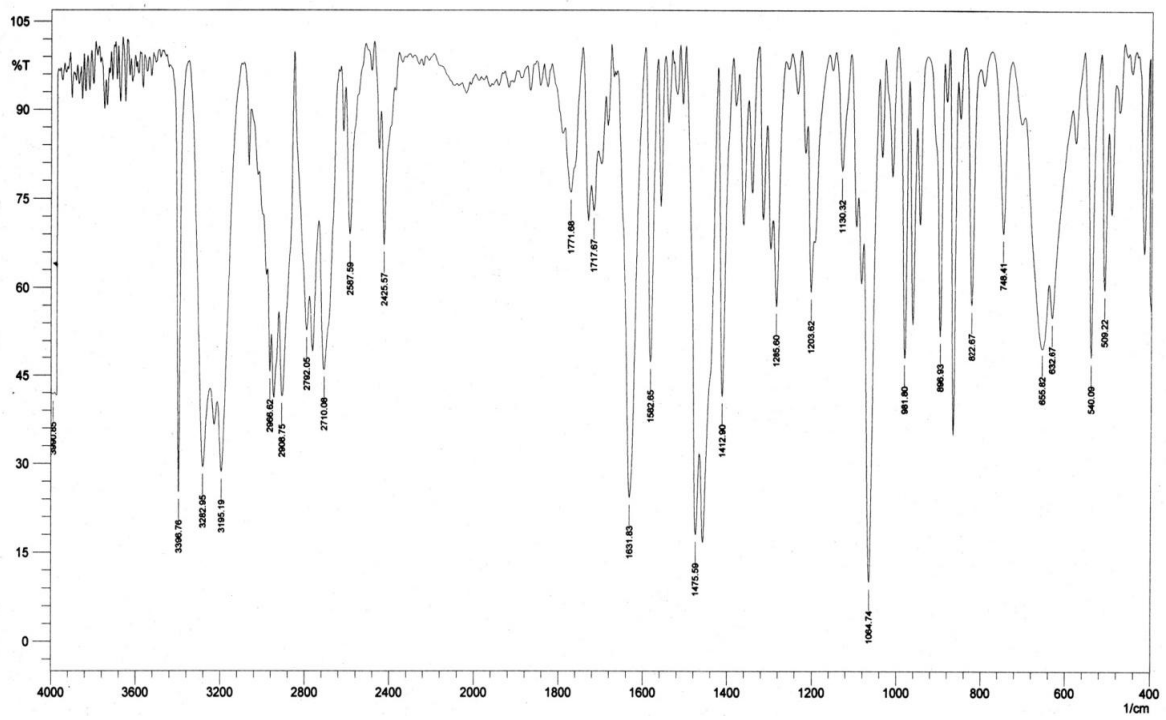


FIG - 96 OVERLAIN SPECTRA OF DESLORATADINE AND AMBROXOL HYDROCHLORIDE

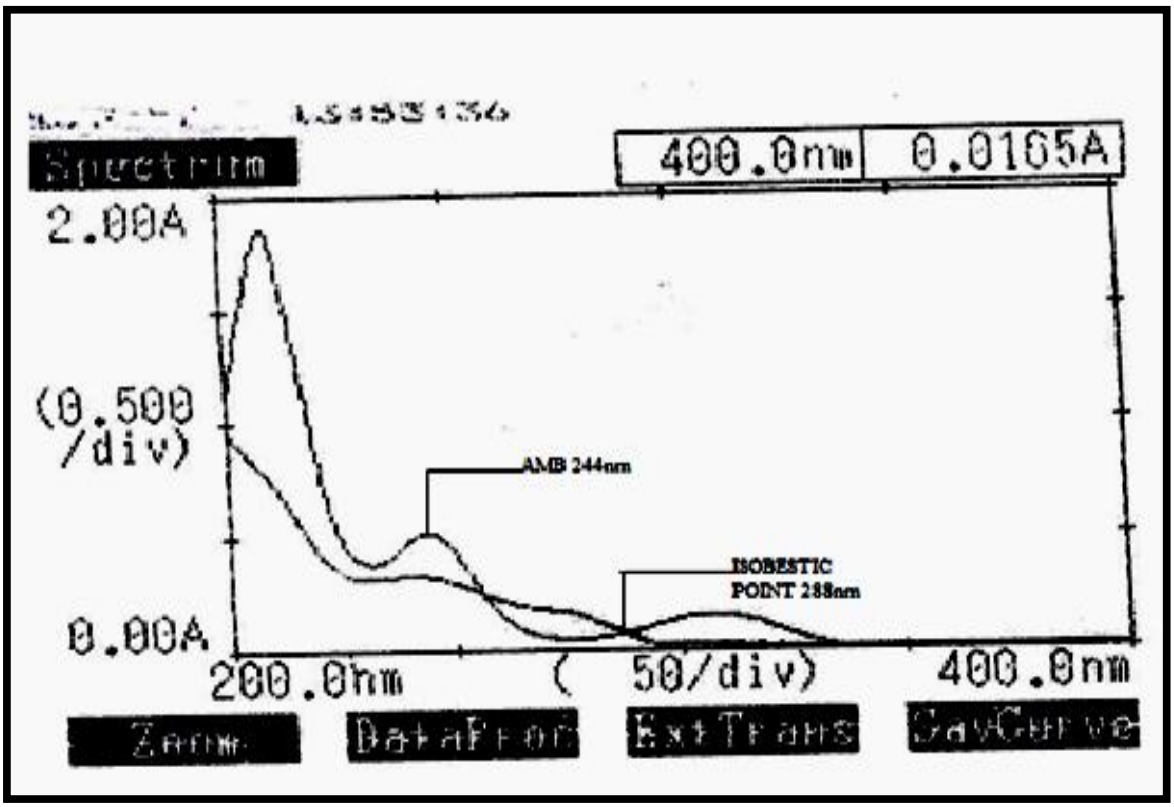


FIG 97 – FIRST ORDER DERIVATIVE SPECTRA OF DESLORATADINE AND AMBROXOL HYDROCHLORIDE

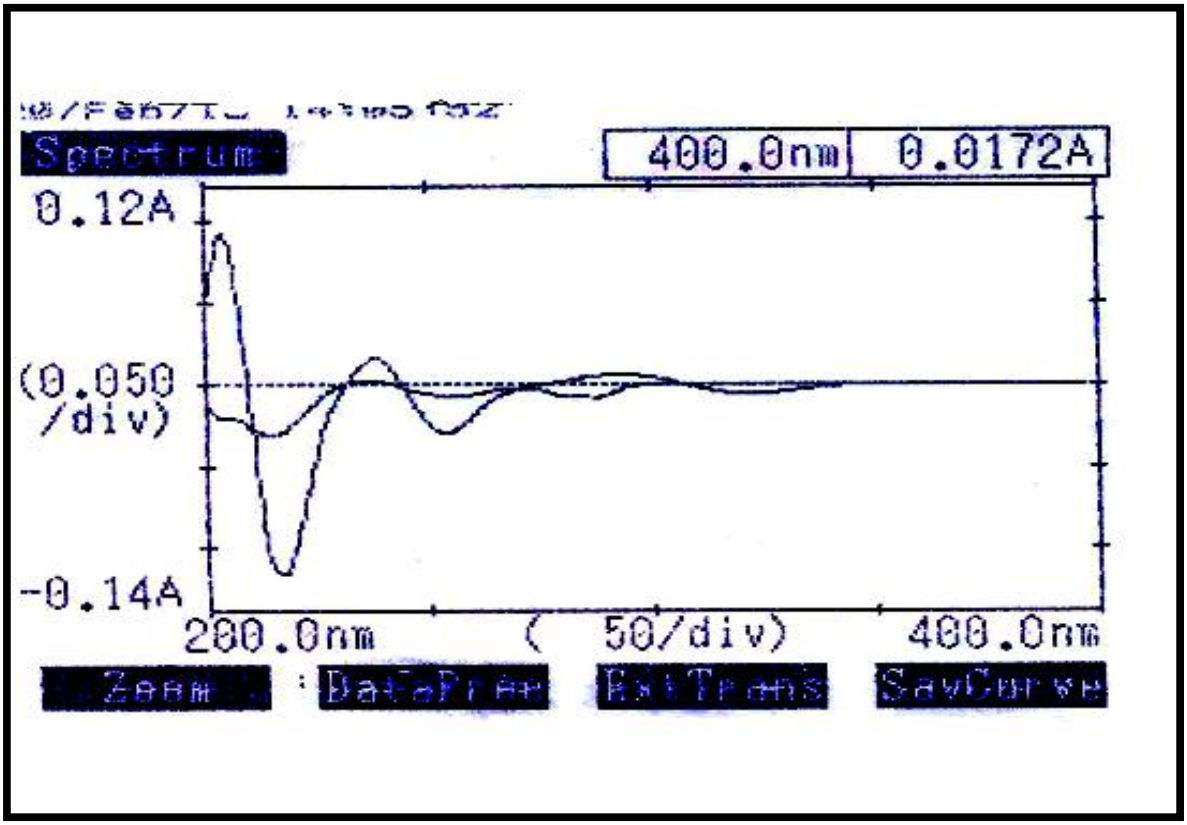


FIG - 98 CALIBRATION CURVE OF AMBROXOL HYDROCHLORIDE At 244nm (ABSORPTION RATIO METHOD)

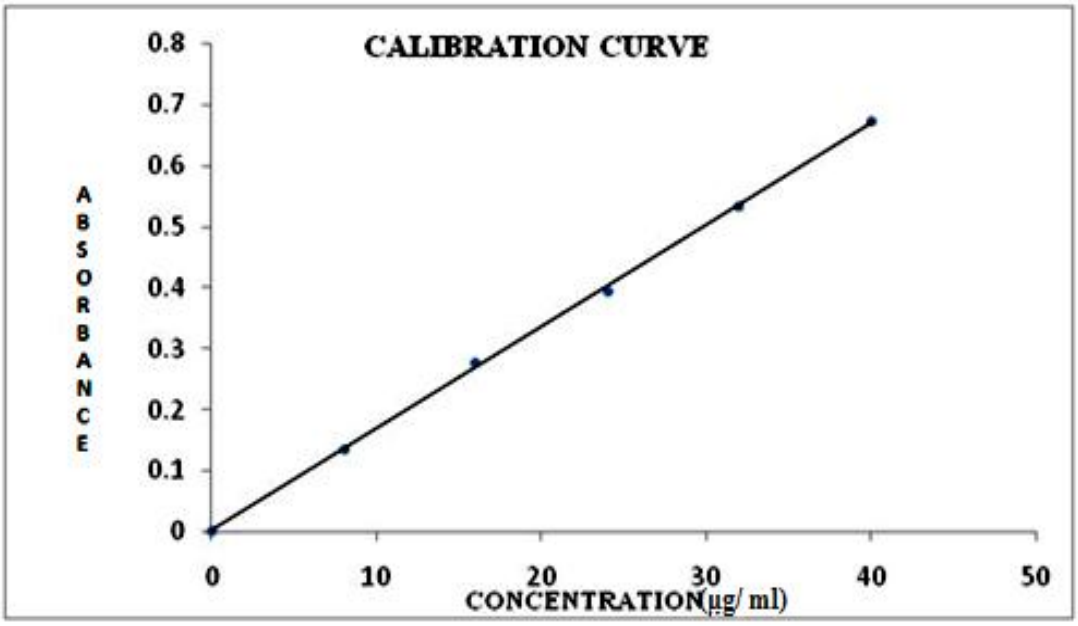


FIG - 99 CALIBRATION CURVE OF AMBROXOL HYDROCHLORIDE

At 288 nm (ABSORPTION RATIO METHOD)

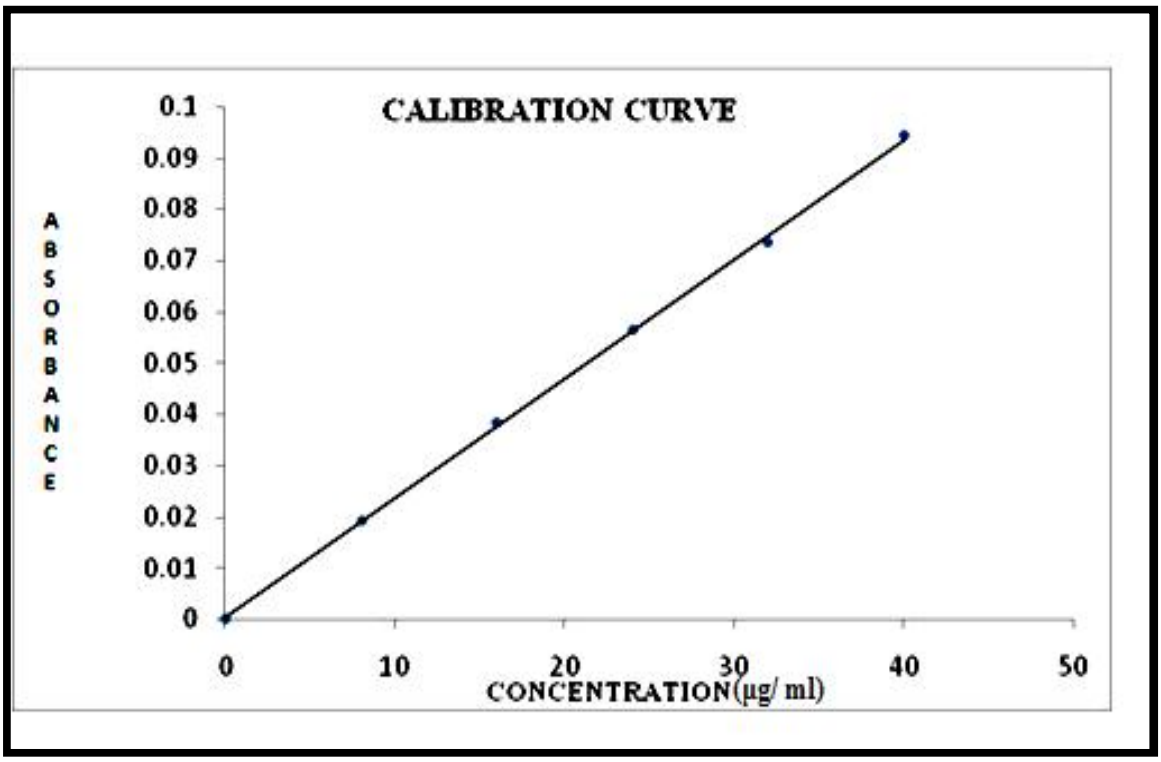


FIG – 100 CALIBRATION CURVE OF DESLORATADINE at 288 nm

(ABSORPTION RATIO METHOD)

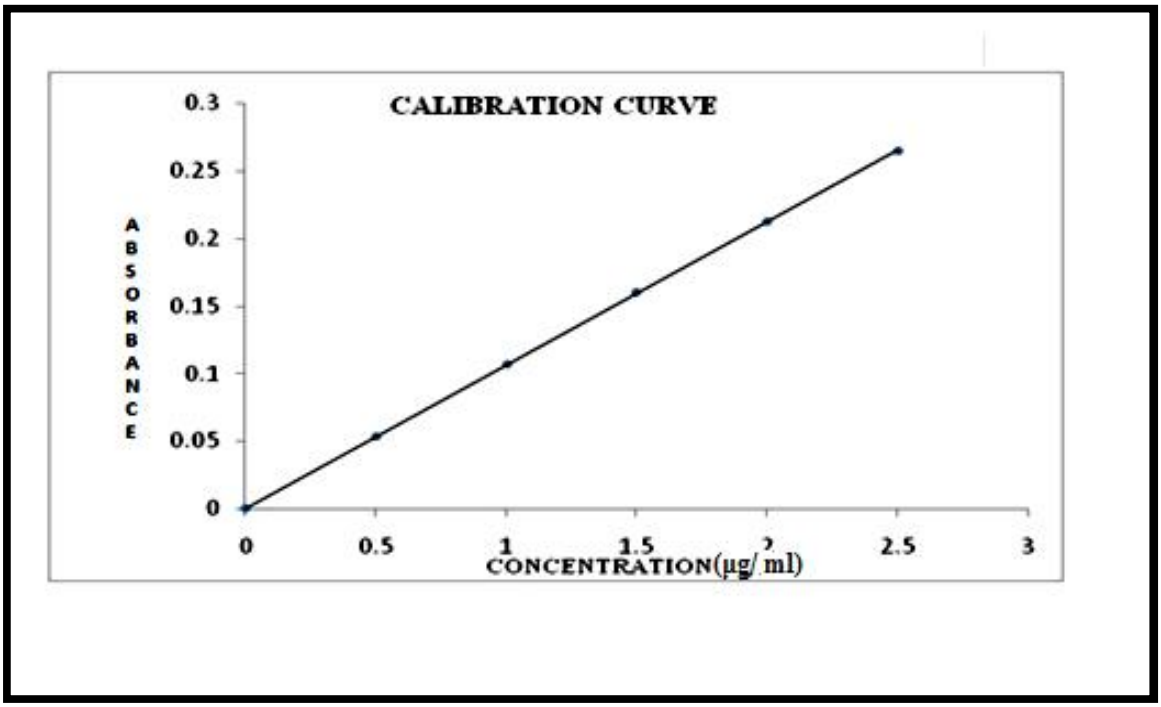


FIG – 101 CALIBRATION CURVE OF DESLORATADINE at 244 nm

(ABSORPTION RATIO METHOD)

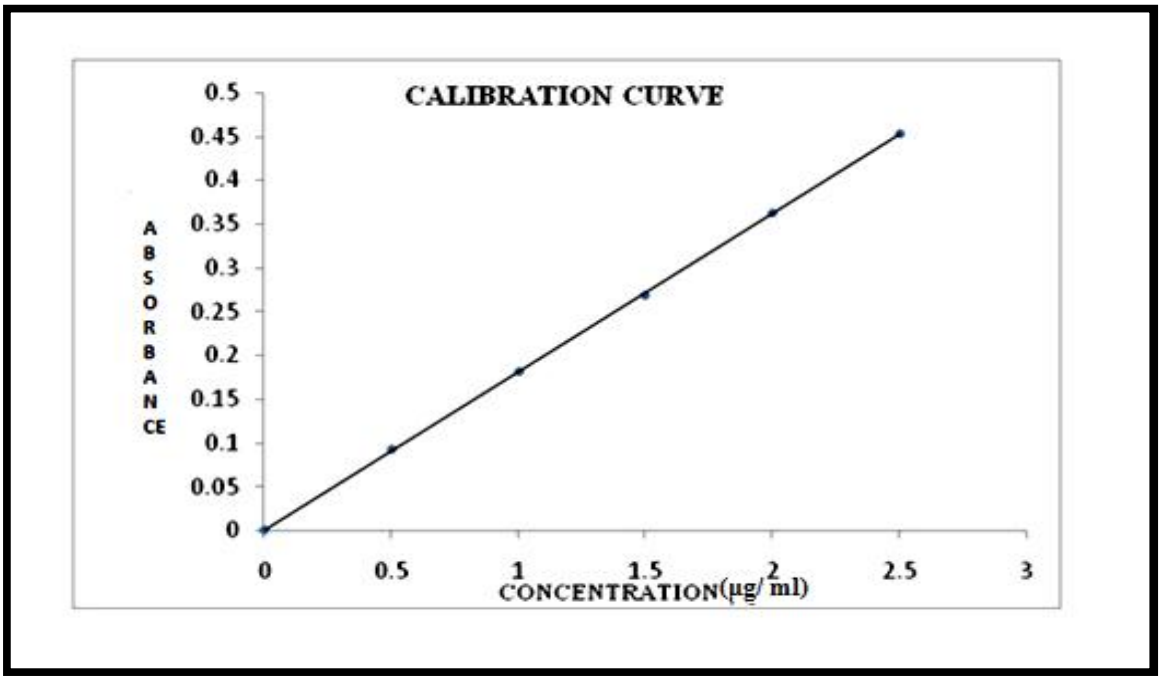


FIG – 102 CALIBRATION CURVE OF DESLORATADINE at 277 nm

(DERIVATIVE METHOD)

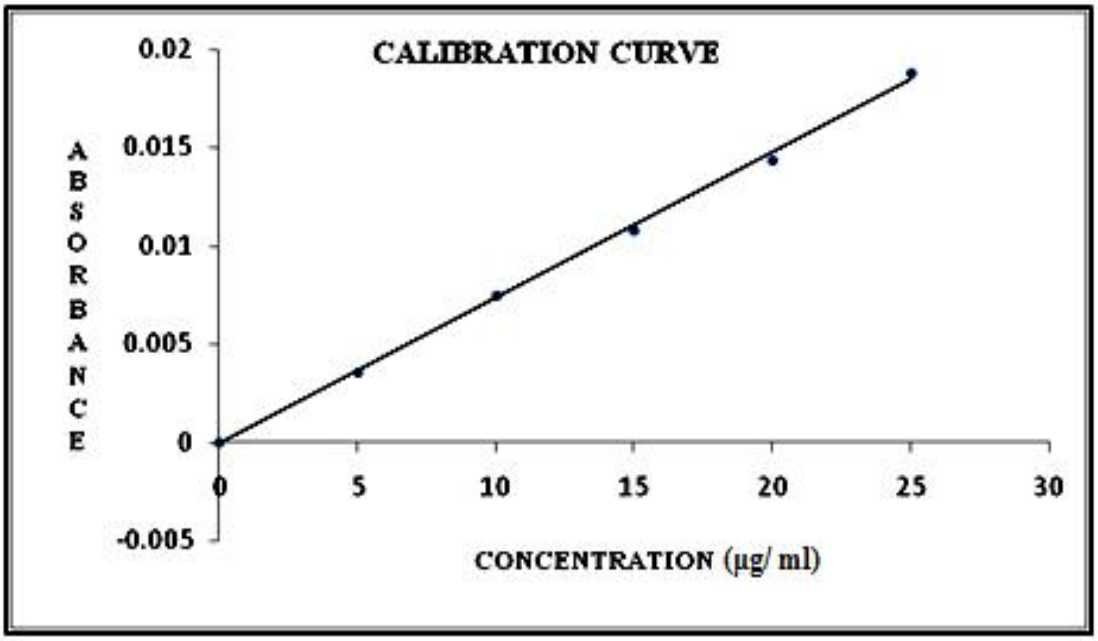


FIG – 103 CALIBRATION CURVE OF AMBROXOL HYDROCHLORIDE

At 320nm (DERIVATIVE METHOD)

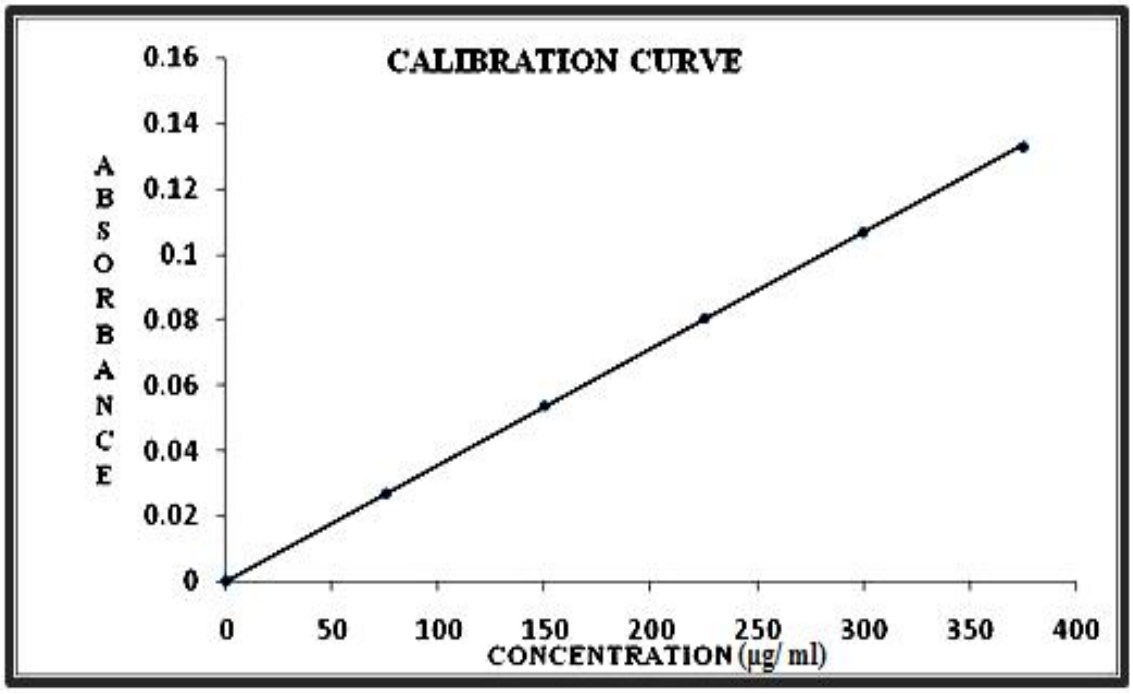


FIG-104 IR SPECRA OF SALBUTAMOL SULPHATE

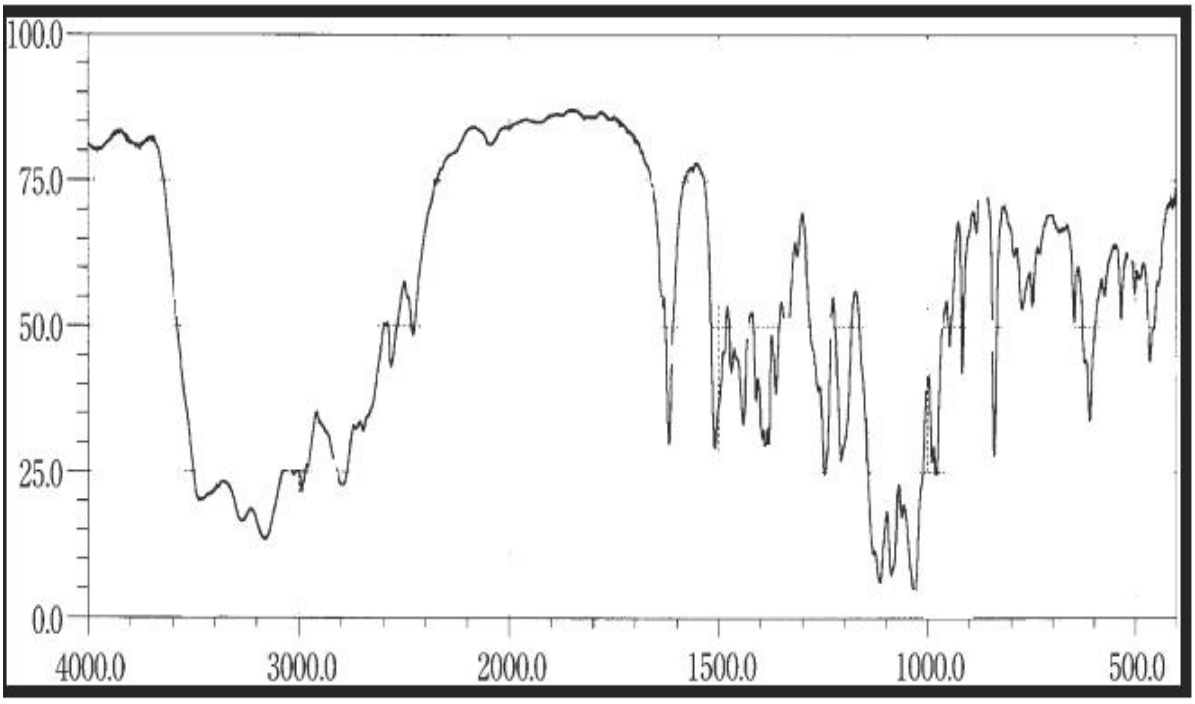


FIG-105 IR SPECRA OF DOXOPHYLLINE

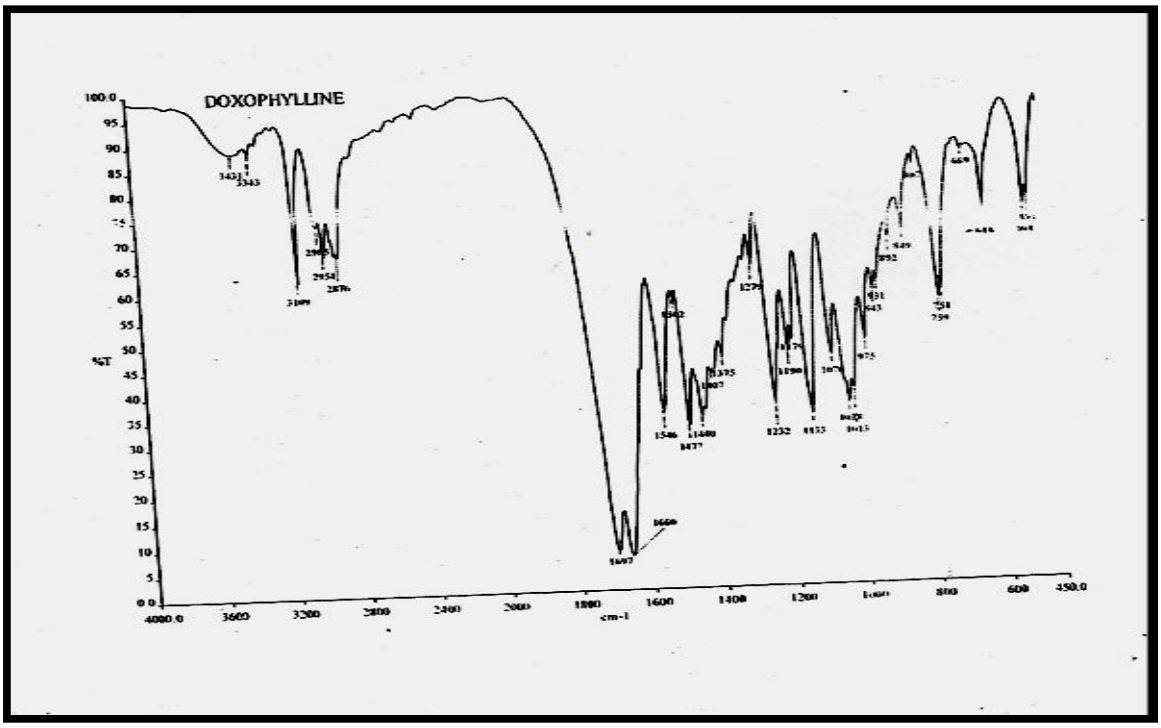


FIG – 106 OVERLAIN SPECTRA OF DOXOFYLLINE AND SALBUTAMOL SULPHATE

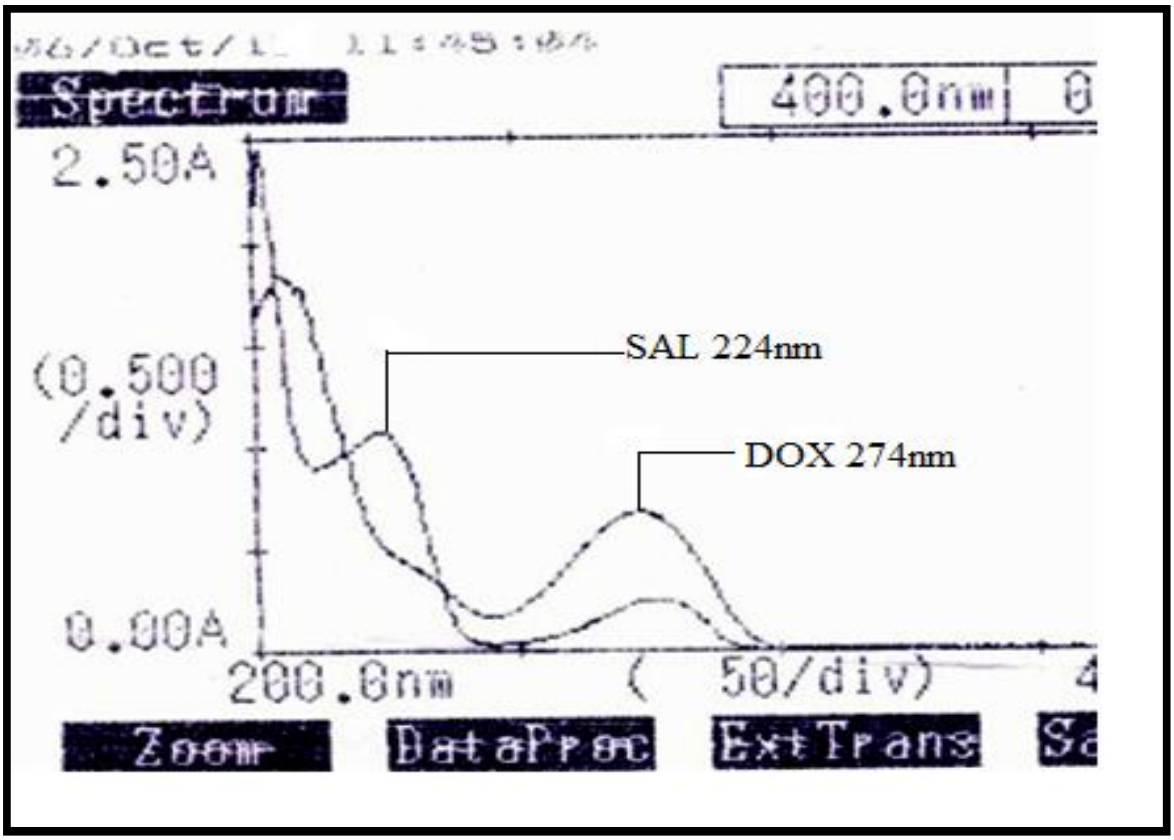


FIG – 107 SECOND ORDER DERIVATIVE SPECTRA OF DOXOFYLLINE AND SALBUTAMOL SULPHATE

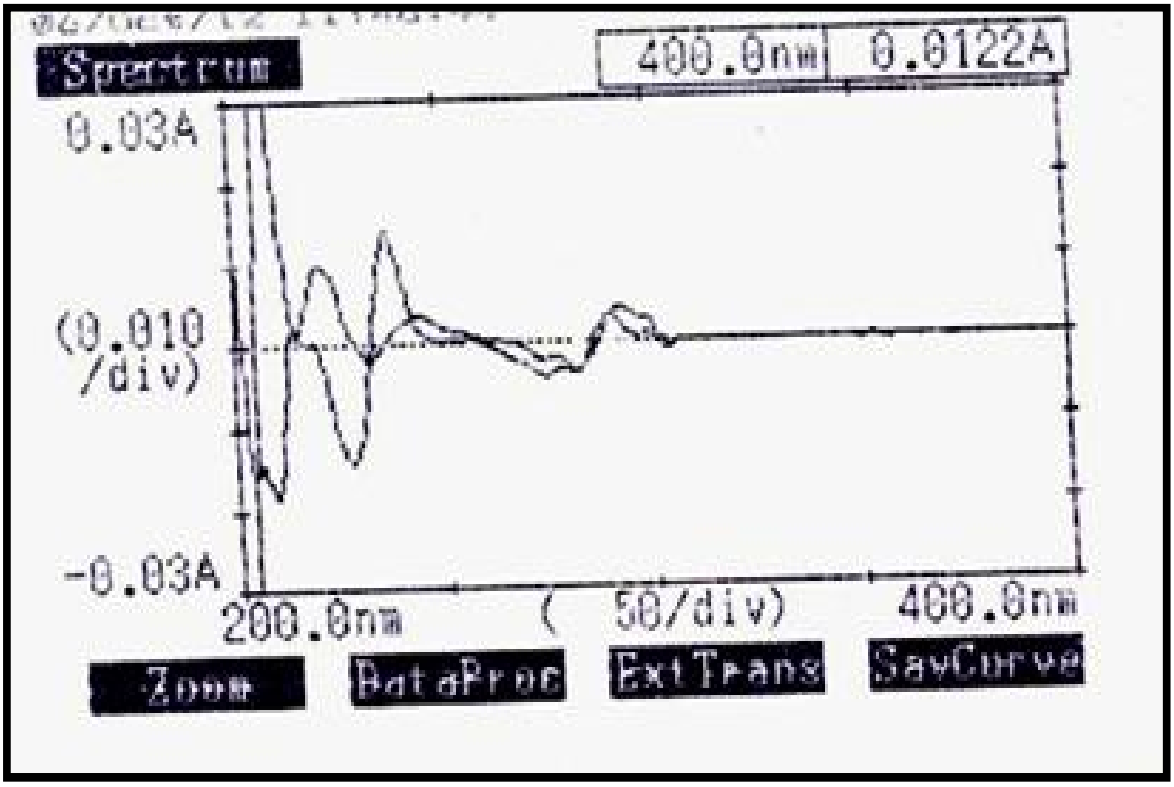


FIG – 108 CALIBRATION CURVE OF DOXOFYLLINE AT 224nm

(SIMULTANEOUS EQUATION METHOD)

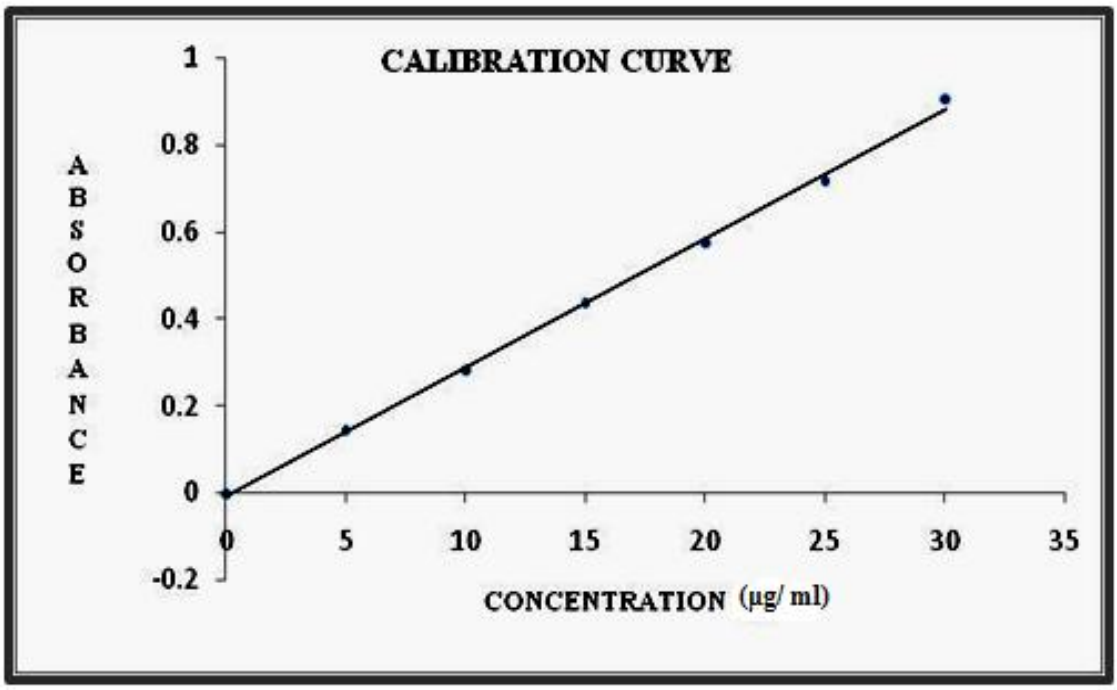


FIG – 109 CALIBRATION CURVE OF DOXOFYLLINE AT 274nm (SIMULTANEOUS EQUATION METHO)

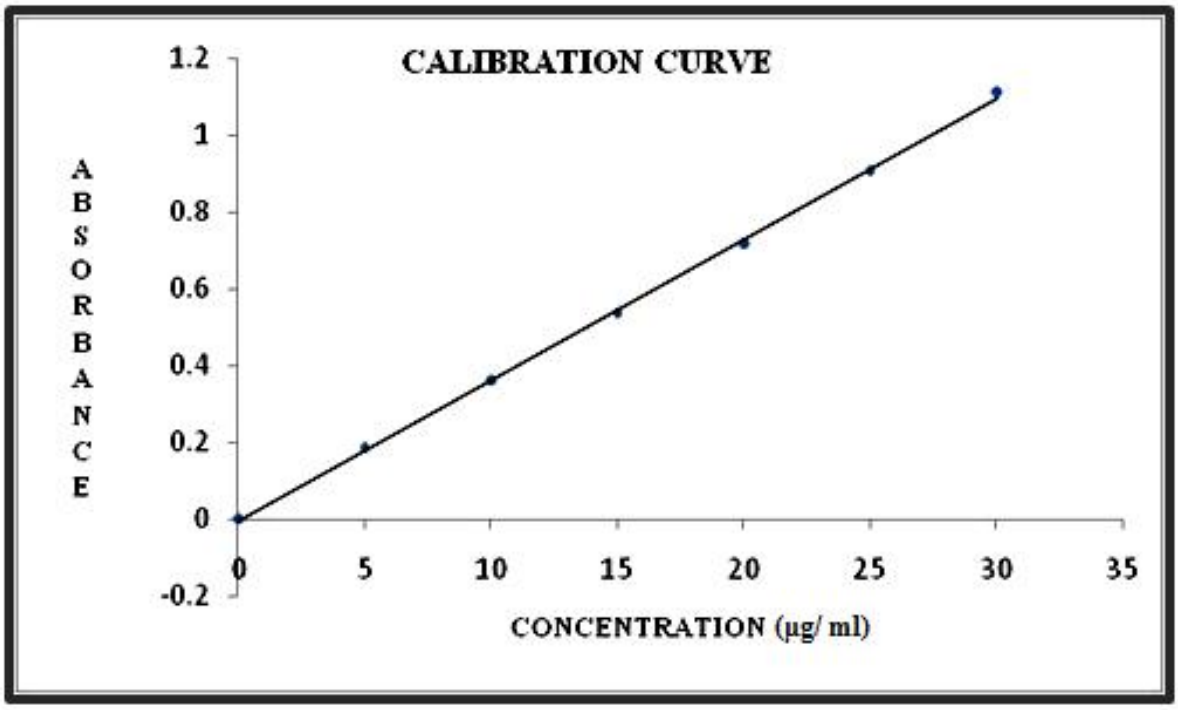


FIG – 110 CALIBRATION CURVE OF SALBUTAMOL SULPHATE AT 224nm (SIMULTANEOUS EQUATION METHOD)

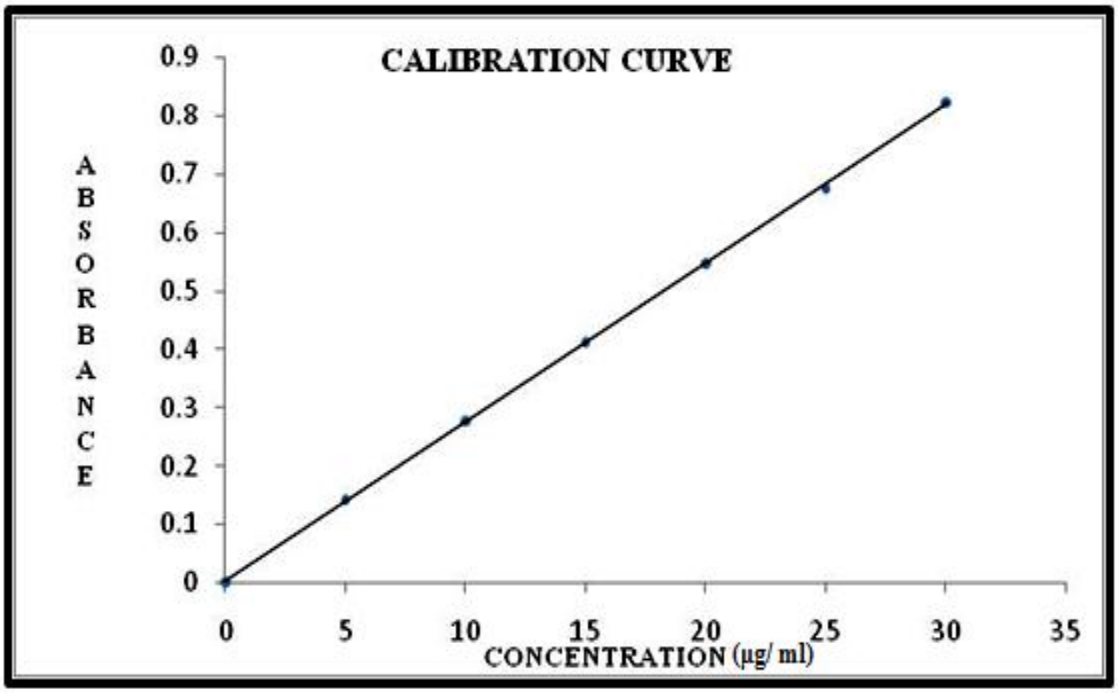


FIG – 111 CALIBRATION CURVE OF SALBUTAMOL SULPHATE AT 274 nm (SIMULTANEOUS EQUATION METHOD)

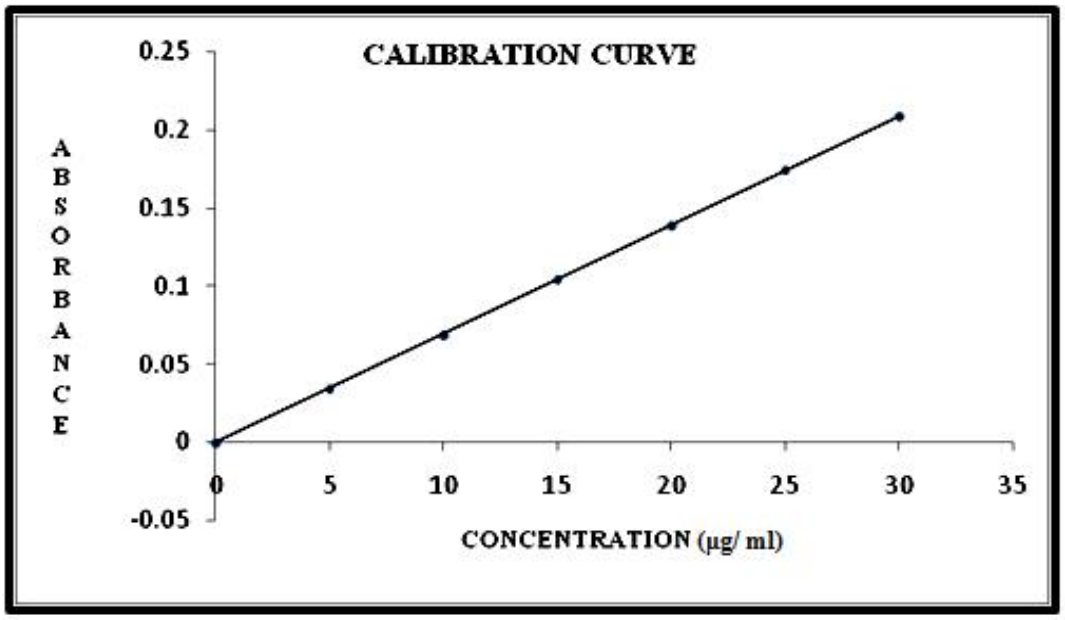


FIG – 112 CALIBRATION CURVE OF DOXOFYLLINE AT 220 - 230 nm (AREA UNDER CURVE METHOD)

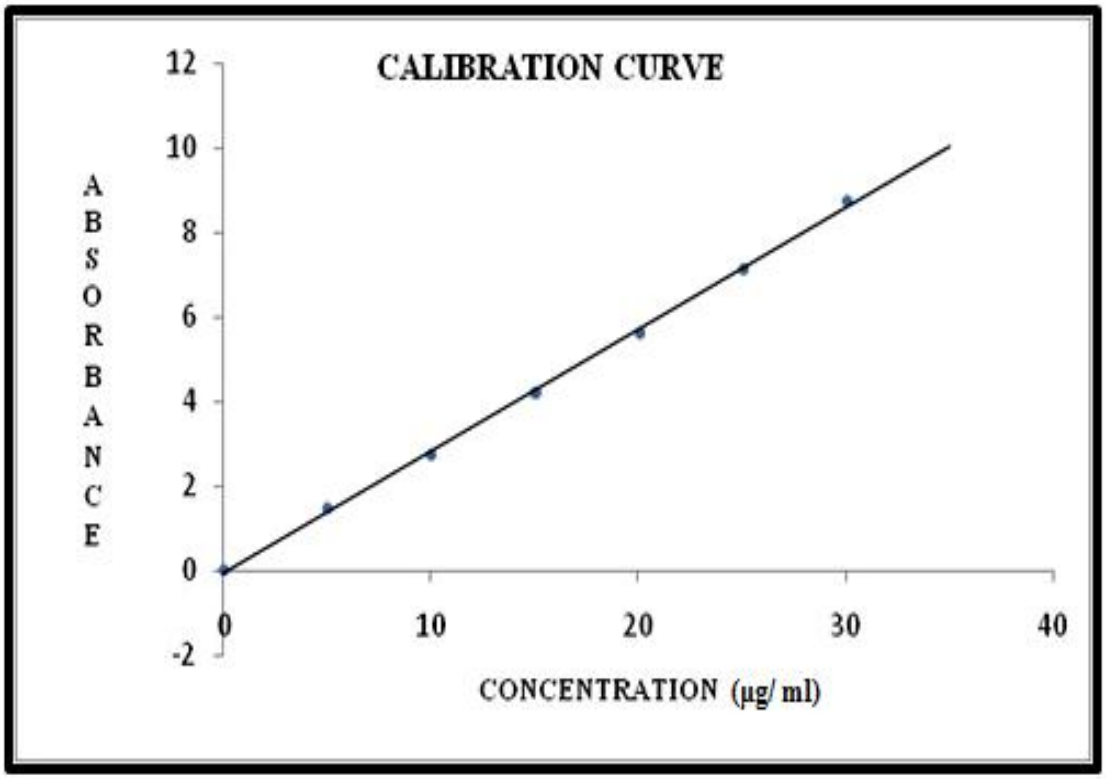


FIG –113 CALIBRATION CURVE OF DOXOFYLLINE AT 270 - 280 nm

(AREA UNDER CURVE METHOD)

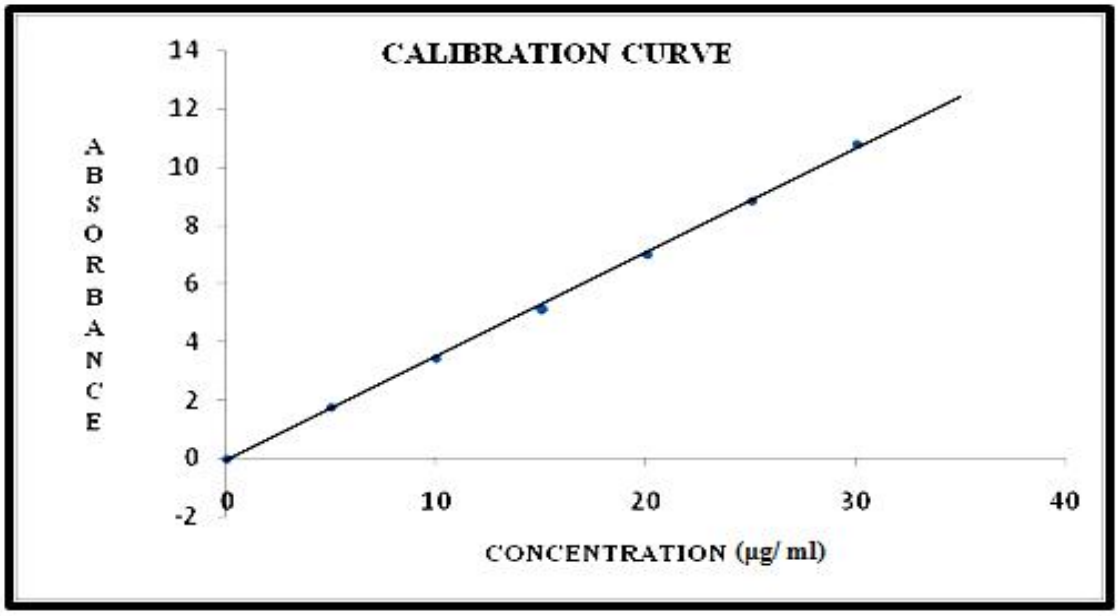


FIG – 114 CALIBRATION CURVE FOR SALBUTAMOL SULPHATE 220 - 230 nm (AREA UNDER CURVE METHOD)

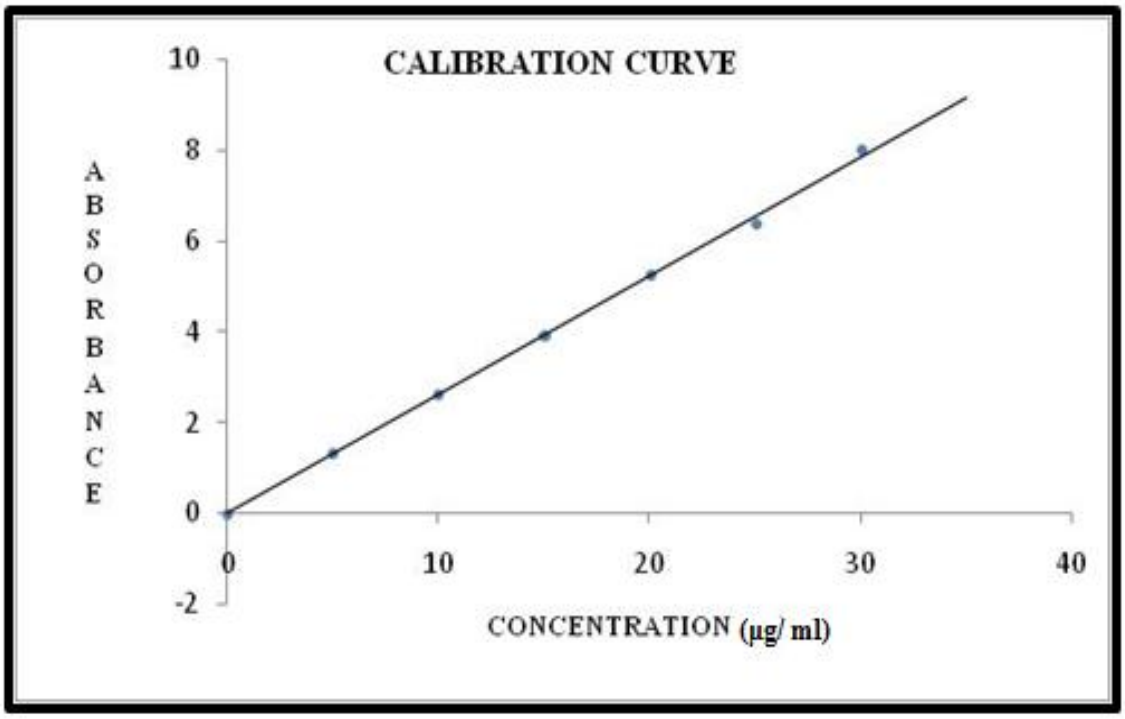


FIG – 115 CALIBRATION CURVE FOR SALBUTAMOL SULPHATE 270 - 280 nm (AREA UNDER CURVE METHOD)

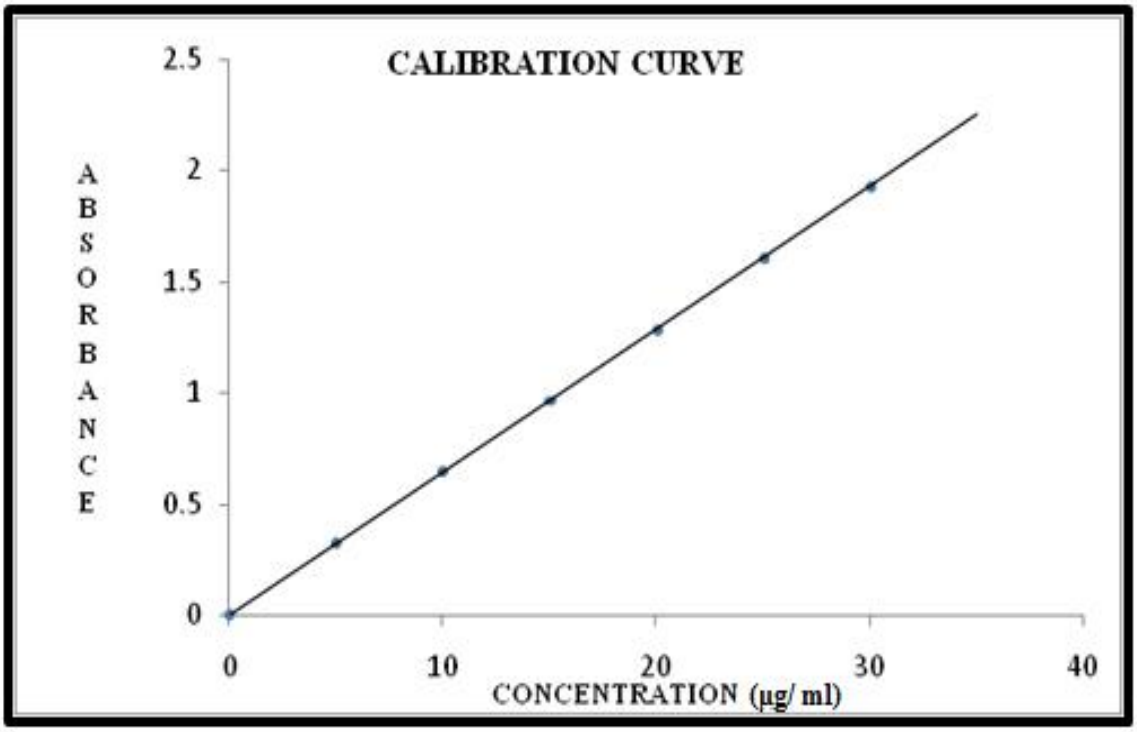


FIG – 116 CALIBRATION CURVE FOR SALBUTAMOL SULPHATE

(DERIVATIVE METHOD)

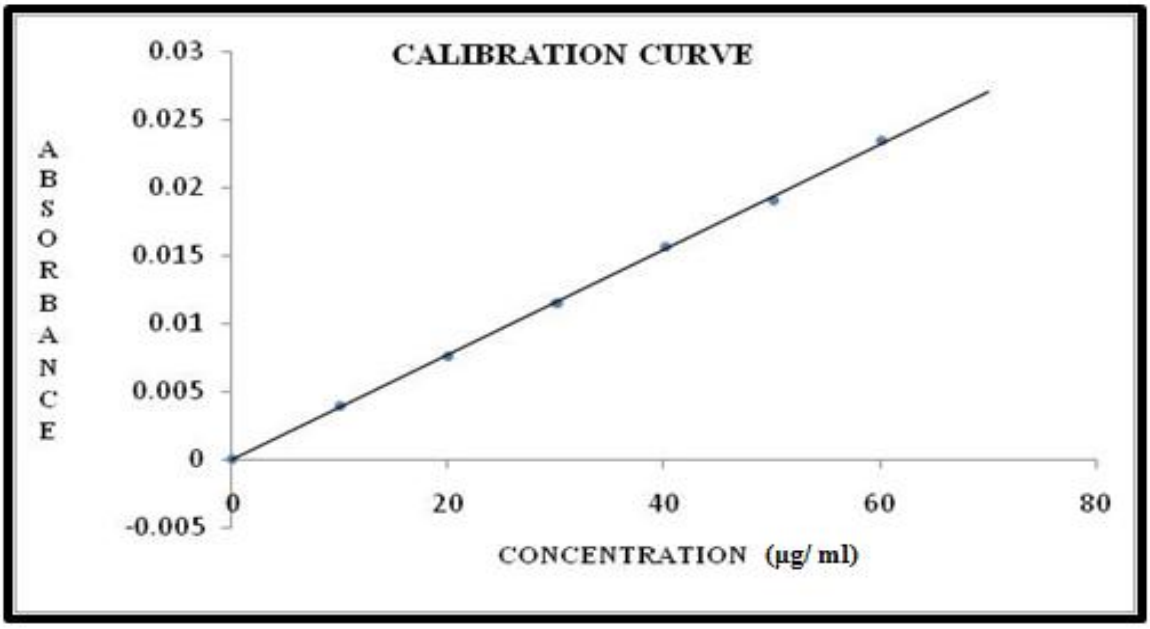
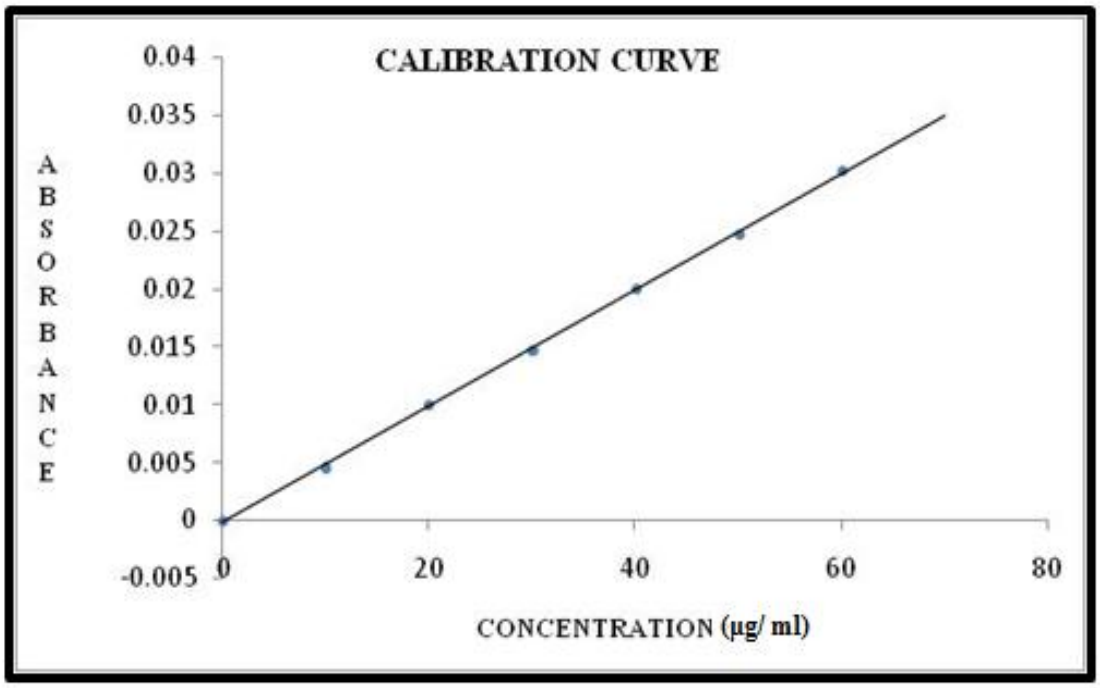


FIG – 117 CALIBRATION CURVE FOR DOXOFYLLINE AT 229 nm (DERIVATIVE METHOD)



TABLES

TABLE-1 SOLUBILITY PROFILE OF TOLPERISONE HYDROCHLORIDE

S.NO	SOLVENT	EXTENT OF SOLUBILTY	CATEGORY
1.	Distilled water	10 mg in 10 µL	Soluble
2.	0.1 M NaOH	10 mg in30 µL	Soluble
3.	0.1 M HCl	10 mg in 10 µL	Soluble
4.	Acetonitrile	10 mg in 3 ml	Slightly soluble
5.	Acetone	10 mg in 60 µL	Freely soluble
6.	Benzene	10 mg in more than 100 ml	Insoluble
7.	Chloroform	10 mg in 7 ml	Slightly soluble
8.	Carbon tetra chloride	10 mg in more than 100 ml	Insoluble
9.	Cyclohexane	10 mg in more than 100 ml	Insoluble
10.	Dimethyl form amide	10 mg in 10 µL	Freely soluble
11.	Diethyl amine	10 mg in 60 µL	Freely soluble
12.	Dichloromethane	10 mg in 6 ml	Sparingly soluble
13.	Ethanol	10 mg in 0.6 ml	Sparingly soluble
14.	Isopropyl alcohol	10 mg in 20 ml	Very slightly soluble
15.	Methanol	10 mg in 0.08 ml	Freely soluble
16.	N-Butanol	10 mg in 8 ml	Slightly soluble
17.	N-Hexane	More than 100 ml	Practically insoluble
18.	Petroleum Spirit	More than 100 ml	Practically insoluble
19.	Alkaline Borate buffer pH 9	10 mg in 7ml	Insoluble
20.	Acid phthalate buffer pH 3	10 mg in 1 ml	Sparingly soluble
21.	Neutralized phthalate buffer pH 3	10 mg in 5 ml	Slightly soluble

TABLE - 2 SOLUBILITY PROFILE OF PARACETAMOL

S.NO	SOLVENT	EXTENT OF SOLUBILTY	CATEGORY
1.	Distilled water	10 mg in 20 µL	Soluble
2.	0.1 M NaOH	10 mg in 30 µL	Soluble
3.	0.1 M HCl	10 mg in 40 µL	Soluble
4.	Acetonitrile	10 mg in 1ml	Sparingly soluble
5.	Acetone	10 mg in 0.06 ml	Freely soluble
6.	Benzene	10 mg in more than10ml	Insoluble
7.	Chloroform	10 mg in more than3ml	Slightly soluble
8.	Pyridine	10 mg in 300 µL	Soluble
9.	Cyclohexane	10 mg in more than3ml	Insoluble
10.	Dim ethyl form amide	10 mg in 150 µL	Freely soluble
11.	Diethyl amine	10 mg in 60 µL	Freely soluble
12.	Dichloromethane	10 mg in 60 µL	Sparingly soluble
13.	Ethanol	10 mg in 0.6 ml	Sparingly soluble
14.	Isopropyl alcohol	10 mg in 1ml	Sparingly soluble
15.	Methanol	10 mg in 200 µL	Freely soluble
16.	N-Butanol	10 mg in 8 ml	Slightly soluble
17.	N-Hexane	10 mg in more than 100 ml	Practically insoluble
18.	Toluene	10 mg in more than 100 ml	Insoluble
19.	Alkaline Borate buffer pH 9	10 mg in 2 ml	Slightly soluble
20.	Acid phthalate buffer pH 3	10 mg in 1 ml	Sparingly soluble
21	Neutralized phthalate buffer pH 3	10 mg in 2 ml	Slightly soluble

TABLE - 3 OPTICAL CHARACTERISTICS OF TOLPERISONE HYDROCHLORIDE (SIMULTANEOUS EQUATION METHOD)

PARAMETERS	**AT 261 nm**	**AT 243 nm**
Beer's law limit (µg/ml)	0.5 – 2.5	0.5 – 2.5
Molar absorptivity (L mol^{-1} $cm^{-1)}$	17720.8416	8116.97964
Sand ell's sensitivity (µg/cm^2/0.001 A.U)	0.01587877	0.35098275
Correlation coefficient (r)	0.999846	0.9996385
Régression équation (Y = mx+c)	Y= 0.06297714x - 0.000104762)	Y= 0.0284914x + 0.00017238
Slope (m)	0.06297714	0.0284914
Intercept (c)	0.00010476	0.00017238
LOD (µg/ml)	0.8457855	0.007044237
LOQ (µg/ml)	2.56299	0.21346143
Standard error	0.000364365	0.00025335

TABLE - 4 OPTICAL CHARACTERISTICS OF PARACETAMOL

(SIMULTANEOUS EQUATION METHOD)

PARAMETERS	**AT 261 nm**	**AT 243 nm**
Beer's law limit (µg/ml)	3-15	3-15
Molar absorptivity (L mol^{-1} $cm^{-1)}$	5683.616	11034.68
Sand ell's sensitivity (µg/cm^2/0.001 A.U)	0.02717321	0.0137006
Correlation coefficient (r)	0.999829	0.99952
Regression equation (Y= mx+c)	Y= 0.03680095x +0.001942857	Y= 0.07299231x + 0.0014110925
Slope (m)	0. 03680095	0.0729923122036
Intercept (c)	0. 001942857	0.001411045
LOD (µg/ ml)	0.3872515	0.02261914
LOQ (µg/ ml)	1.173497	0.0685427
Standard error	0.004270095	0.001411045

TABLE - 5 SYNTHETIC MIXTURES (SIMULTANEOUS EQUATION METHOD)

Drug	Sample No.	Cocentration (µg/ ml)	Amount found (µg/ ml)	Percentage obtained	Average (%)	S.D	% R.S.D.	S.E.
TPE	1	0.5	0.5020	100.04	100.104	0.22952	0.22928	0.00918
	2	1	0.9998	99.98				
	3	1.5	1.4988	99.92				
	4	2	2.010	100.5				
	5	2.5	2.5021	100.08				
PCL	1	3	3.011	100.36	100.1002	0.236844	0.236607	0.009474
	2	6	5.989	99.81				
	3	9	9.021	100.23				
	4	12	11.987	99.891				
	5	15	15.032	100.21				

TABLE – 6 QUANTIFICATION OF FORMULATION (SIMULTANEOUS EQUATION METHOD)

Drug	Sample No.	Labeled amount (mg/ tab)	Amount found (mg/ tab)*	Percentage obtained*	Average (%)	S.D	% R.S.D.	S.E.
TPE	1	50	49.75	99.50	99.558	0.48602	0.488219	0.01350
	2	50	50.04	100.01				
	3	50	49.83	99.60				
	4	50	49.35	98.7				
	5	50	49.75	99.5				
	6	50	50.07	100.04				
PCL	1	300	300.30	100.1	100.0183	0.04167	0.41666	0.01158
	2	300	300.01	100.00				
	3	300	299.97	99.99				
	4	300	300.06	100.02				
	5	300	300.04	100.01				
	6	300	299.97	99.99				

* Mean of Six Observations

TABLE– 7 INTRA DAY AND INTER DAY ANALYSIS OF FORMULATION

(SIMULTANEOUS EQUATION 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
TPE	1	50	101.6666	101.0000	0.5092	0.3363	0.5030	0.3301
	2	50	101.3333	100.0120				
	3	50	100.6666	100.6606				
Mean			**101.5555**	**100.9979**				
PCL	1	300	98.0000	98.0000	0.1928	0.3330	0.1969	0.3386
	2	300	98.0000	98.3333				
	3	300	97.6666	98.6666				
Mean			**97.8888**	**98.3333**				

* Mean of Three Observations

TABLE - 8 RUGGEDNESS STUDY (SIMULTANEOUS EQUATION METHOD)

Drug	Condition	% Obtained	S.D	%R.S.D	S.E
TPE	Analyst 1	101.6111	1.1818	1.1669	0.0328
	Analyst 2	100.3333	1.1739	1.1584	0.0326
	Instrument 1	101.1666	0.5476	0.5413	0.0152
	Instrument 2	100.5551	1.2047	1.1863	0.0334
PCL	Analyst 1	98.3888	0.9291	0.9443	0.0258
	Analyst 2	98.1666	0.5868	0.5978	0.0163
	Instrument 1	98.2777	0.4907	0.4993	0.0136
	Instrument 2	98.6666	0.4216	0.4273	0.0117

TABLE – 9 RECOVERY ANALYSIS OF FORMULATION (SIMULTANEOUS EQUATION METHOD)

Drug	Sample No.	Amount present (µg/ ml)	Amount added (µg/ ml)	Amount estimated (µg/ ml)	Amount recovered (µg/ ml)	% Recovery	S.D	% R.S.D	S.E.
	1	1.5010	1.2	2.6857	1.1847	98.72			
TPE	2	1.5010	1.5	3.010	1.509	100.53	1.2230	1.2218	0.1358
	3	1.5010	1.8	3.3200	1.819	101.05			
					Mean	**100.1**			
	1	8.999	7.2	16.2280	7.229	100.03			
PCL	2	8.999	9.0	18.0141	9.0151	100.16	0.08144	0.08139	0.0090
	3	8.999	10.8	19.8021	10.8021	100.01			
					Mean	**100.06**			

TABLE – 10 OPTICAL CHARACTERISTICS OF TOLPERISONE HYDROCHLORIDE (ABSORPTION RATIO METHOD)

PARAMETERS	**AT 254 nm**	**AT 243 nm**
Beer's law limit (µg/ml)	0.5 – 2.5	0.5 – 2.5
Molar absorptivity (L mol^{-1} $cm^{-1)}$	12963.72	8116.416
Sand ell' sensitivity (µg/cm^2/0.001A.U)	0.21628302	0.35098275
Correlation coefficient (r)	0.99997	0.9996385
Regression équation (Y = mx +c)	Y = 0.04623x - 8.212429E-05	Y = 0.0284914x + 0.00017238
Slope (m)	0.04623	0.0284914
Intercept (c)	8.212429E-05	0.00017238
LOD (µg/ml)	0.0020741	0.00017238
LOQ (µg/ml)	0.006285	0.21346143
Standard error	0.00013065	0.00025335

TABLE – 11 OPTICAL CHARACTERISTICS OF PARACETAMOL

(ABSORPTION RATIO METHOD)

PARAMETERS	AT 254 nm	AT 243 nm
Beer's law limit (µg/ml)	3-15	3-15
Molar absorptivity (L mol^{-1} $cm^{-1)}$	8581.4656	11034.68
Sand ell's sensitivity (µg/cm^2/0.001 A.U)	0.0176263	0.0137006
Correlation coefficient (r)	0.99998	0.99952
Regression equation (Y = mx+c)	Y= 0.056733x - 0.001428571)	Y = 0.07299231x + 0.0014110925
Slope (m)	0.056733	0.0729923122036
Intercept (c)	- 0.001428571	0.0014110925
LOD (µg/ml)	0.113188	0.02261914
LOQ (µg/ml)	0.34299	0.0685427
Standard error	0.00195725	0.001411045

TABLE – 12 SYNTHETIC MIXTURES (ABSORPTION RATIO METHOD)

Drug	Sample No.	Concentration (µg/ ml)	Amount found (µg/ ml)	Percentage obtained	Average (%)	S.D	% R.S.D.	S.E.
TPE	1	0.5	0.4999	99.98	99.99	0.42083	0.42087	0.01683
	2	1	1.0054	100.54				
	3	1.5	1.5023	100.15				
	4	2	1.9876	99.38				
	5	2.5	2.4976	99.90				
PCL	1	3	3.021	100.70	100.266	0.43483	0.43368	0.01739
	2	6	6.041	100.68				
	3	9	8.9998	99.99				
	4	12	12.032	100.26				
	5	15	14.9563	99.70				

TABLE – 13 QUANTIFICATION OF FORMULATION (ABSORPTION RATIO METHOD)

Drug	Sample No.	Labeled amount (mg/ tab)	Amount found (mg/ tab)	Percentage obtained	Average (%)	S.D	% R.S.D.	S.E.
	1	50	49.00	98.00				
	2	50	49.66	99.32				
TPE	3	50	49.88	99.76	98.971	0.87395	0.88305	0.02427
	4	50	49.34	98.68				
	5	50	49.01	98.02				
	6	50	50.02	100.04				
	1	300	299.05	99.68				
	2	300	300.01	100.00				
PCL	3	300	300.03	100.01	99.88	0.16940	0.16959	0.00470
	4	300	299.98	99.99				
	5	300	299.98	99.99				
	6	300	299.00	99.66				

TABLE – 14 INTRA DAY AND INTER DAY ANALYSIS OF FORMULATION (ABSORPTION RATIO 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
TPE	1	50	99.4590	99.3450	0.167633	0.51079	0.1687	0.5132
	2	50	99.1340	99.1236				
	3	50	99.3678	100.098				
		Mean	**99.3202**	**99.5222**				
PCL	1	300	99.3516	99.7878	0.4581	0.1780	0.4588	0.1783
	2	300	99.9808	100.0100				
	3	300	100.2431	99.6580				
		Mean	**99.8585**	**99.8186**				

* Mean of Three Observations

TABLE – 15 RUGGEDNESS STUDY (ABSORPTION RATIO METHOD)

Drug	Condition	% Obtained	S.D	%R.S.D	S.E
TPE	Analyst 1	99.73	0.12289	0.1232	0.0136
	Analyst 2	99.166	0.5982	0.6032	0.0664
	Instrument 1	100.61	0.8400	0.8414	0.09334
	Instrument 2	99.87	0.2810	0.2820	0.0312
PCL	Analyst 1	98.986	0.36225	0.3604	0.04025
	Analyst 2	99.456	0.4196	0.41766	0.046626
	Instrument 1	100.13	0.220377	0.22076	0.07528
	Instrument 2	100.03	0.0500	0.04999	0.0055

TABLE – 16 RECOVERY ANALYSIS OF FORMULATION (ABSORPTION RATIO METHOD)

Drug	Sample No.	Amount present (µg/ ml)	Amount added (µg/ ml)	Amount estimated* (µg/ ml)	Amount recovered (µg/ ml)	% Recovery*	S.D	% R.S.D	S.E.
TPE	1	1.5010	1.2	2.6994	1.1984	99.86	0.23692	0.2376	0.02632
	2		1.5	2.9986	1.4976	99.84			
	3	1.5010	1.8	3.2910	1.7901	99.44			
		1.5010							
					Mean	**99.71**			
PCL	1	9.001	7.2	16.1890	7.188	99.830	0.10084	0.100902	0.01120
	2	9.001	9	18.0023	9.0013	100.00			
	3	9.001	10.8	19.8010	10.8010	100.009			
					Mean	**99.94633**			

* Mean of Three Observations

TABLE -17 OPTICAL CHARACTERISTICS OF PARACETAMOL (AREA UNDER CURVE METHOD)

PARAMETERS	**AT 253-269 nm**	**AT 274- 284nm**
Beer's law limit (µg/ml)	3 – 18	3 – 18
Molar absorptivity (L mol^{-1} $cm^{-1)}$	8925.4941	44614.8714
Sand ell's sensitivity (µg/cm^2/0.001 A.U)	0.09645393	0.0805107
Correlation coefficient (r)	0.99965	0.999655
Régression equation (Y = mx+c)	Y= 0.58674285x + 0.0259619	Y = 0.48864857x + 0.021819
Slope (m)	0.58674285	0.48864857
Intercept (c)	0.0259619	0.021819
LOD (µg/ml)	0.1573532	0.143268
LOQ (µg/ml)	0.476823	0.487699
Standard error	0.09645392	0.080510771

TABLE – 18 OPTICAL CHARACTERISTICS OF TOLPERISONE HYDROCHLORIDE (AREA UNDER CURVE METHOD)

PARAMETERS	**AT 253 - 269 nm**	**AT 274 - 284 nm**
Beer's law limit (µg/ml)	0.5 – 2.5	0.5 – 2.5
Molar absorptivity (L mol^{-1} $cm^{-1)}$	52469.247	43451.007
Sand ell's sensitivity(µg/cm^2/0.001 A.U)	0.070729772	0.064850013
Correlation coefficient (r)	0.99921	0.99908
Regression equation (Y = mx+c)	Y = 1.70488x + 0.081942	Y = 1.44694x + 0.057747619
Slope (m)	1.70488	1.44694
Intercept (c)	0.081942	0.057747619
LOD (µg/ml)	0.0262255	0.0265547
LOQ (µg/ml)	0.079471	0.079471355
Standard error	0.000587	0.00069111

TABLE – 19 SYNTHEIC MIXTURES (AREA UNDER CURVE METHOD)

Drug	Sample No.	Concentration (µg/ml)	Amount found(µg/ml)	Percentage obtained*	Average (%)	S.D	% R.S.D.	S.E.
TPE	1	0.5	0.5012	100.24	100.0536	0.59002	0.58971	0.02360
	2	1	1.0079	100.79				
	3	1.5	1.5020	100.13				
	4	2	1.9832	99.16				
	5	2.5	2.4987	99.948				
PCL	1	3	3.021	99.48	99.814	0.2342	0.2347	0.00937
	2	6	6.023	100.00				
	3	9	9.031	100.01				
	4	12	11.987	99.92				
	5	15	15.061	99.66				

TABLE – 20 QUANTIFICATION OF FORMULATION (AREA UNDER CURVE METHOD)

Drug	Sample No.	Labeled amount (mg/ tab)	Amount found (mg/ tab)*	Percentage obtained*	Average (%)	S.D	% R.S.D.	S.E.
TPE	1	50	50.04	100.08	99.83	0.31864	0.319	0.00885
	2	50	49.66	99.32				
	3	50	50.01	100.02				
	4	50	49.78	99.56				
	5	50	50.05	100.01				
	6	50	50.02	100.04				
PCL	1	300	298.45	99.48	99.8466	0.22429	0.22463	0.00623
	2	300	300.01	100.00				
	3	300	300.03	100.01				
	4	300	299.76	99.92				
	5	300	298.98	99.66				
	6	300	300.05	100.01				

* Mean of Six Observations

TABLE – 21 INTRA DAY AND INTER DAY ANALYSIS OF FORMULATION (AREA UNDER 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
TPE	1	50	99.6867	100.01				
	2	50	99.3000	99.960	0.40066	0.0709	0.40193	0.07098
	3	50	100.101	99.87				
		Mean	**99.6867**	**99.9467**				
PCL	1	300	99.700	99.7878				
	2	300	99.30	100.0100	0.4509	0.1780	0.45213	0.1783
	3	300	100.2	99.6580				
		Mean	**99.733**	**99.8186**				

* Mean of Three Observations

TABLE – 22 RUGGEDNESS STUDY (AREA UNDER CURVE METHOD)

Drug	Condition	% Obtained	S.D	% R.S.D	S.E
TPE	Analyst 1	99.8633	0.220377	0.4595	0.0515
	Analyst 2	99.7336	0.6578	0.6604	0.07069
	Instrument 1	100.5053	0.36225	0.3604	0.04025
	Instrument 2	100.474	0.4196	0.41766	0.046626
PCL	Analyst 1	99.83	0.8400	0.8414	0.09334
	Analyst 2	99.6589	0.2810	0.2820	0.0312
	Instrument 1	100.084	0.220377	0.22076	0.02445
	Instrument 2	99.611	0.2136	0.2141	0.0237

TABLE – 23 RECOVERY ANALYSIS OF FORMULATION (AREA UNDER CURVE METHOD)

Drug	Sample No.	Amount present (µg/ ml)	Amount added (µg/ ml)	Amount estimated* (µg/ ml)	Amount recovered (µg/ ml)	% Recovery*	S.D	% R.S.D	S.E.
TPE	1	1.5010	1.2	2.7014	1.2004	100.03	0.79999	0.79908	0.08889
	2	1.5010	1.5	3.0152	1.5142	100.94			
	3	1.5010	1.8	3.2895	1.7885	99.36			
					Mean	**99.688**			
PCL	1	9.001	7.2	16.1923	7.1923	99.879	0.22108	0.22123	0.02456
	2	9.001	9	18.0145	9.0135	100.15			
	3	9.001	10.8	19.7699	10.7699	99.712			
					Mean	**99.91**			

TABLE – 24 OPTICAL CHARACTERISTICS OF TOLPERISONE HYDROCHLORIDE & PARACETAMOL (FIRST ORDER DERIVATIVE METHOD)

PARAMETERS	**TPE at 243 nm**	**PCL at 261 nm**
Beer's law limit (µg/ml)	1 - 5	6 - 30
Molar absorptivity (L mol^{-1} $cm^{-1)}$	816.214	229.7632
Sandell's sensitivity (µg/cm^2/0.001A.U)	0.456338	0.39407018
Correlation coefficient (r)	0.99912	0.99968
Regression equation (Y = mx+c)	Y = 0.00219149x + 0.0001047	Y = 0.00253761x + 0.00013809
Slope (m)	0.00219149	0.00253761
Intercept (c)	+ 0.0001047	0.00013809
LOD (µg/ml)	0.150854	0.984396
LOQ (µg/ml)	0.457136	2.983018
Standard error	0.000197	0.0007916

TABLE – 25 SYNTHETIC MIXTURES (FIRST ORDER DERIVATIVE METHOD)

Drug	Sample No.	Concentration (µg/ ml)	Amount found(µg/ml)	Percentage obtained*	Average (%)	S.D	% R.S.D.	S.E.
TPE	1	1	1.003	100.30	100.544	0.48397	0.48135	0.01935
	2	2	2.011	100.55				
	3	3	2.999	99.96				
	4	4	4.051	101.27				
	5	5	5.032	100.64				
PCL	1	6	6.012	100.20	100.235	0.46793	0.46684	0.02924
	2	12	12.112	100.93				
	3	18	18.002	100.01				
	4	24	24.020	100.08				
	5	30	29.976	99.92				

* Mean of three Observations

TABLE – 26 QUANTIFICATION OF FORMULATION (FIRST ORDER DERIVATIVE METHOD)

Drug	Sample No.	Labeled amount (mg/ tab)	Amount found (mg/ tab)*	Percentage obtained*	Average (%)	S.D	% R.S.D.	S.E.
TPE	1	50	50.01	100.02	100.026	0.06653	0.06651	0.00184
	2	50	49.98	99.96				
	3	50	50.07	100.14				
	4	50	50.02	100.04				
	5	50	49.98	99.96				
	6	50	50.02	100.04				
PCL	1	300	298.0887	99.3629	99.7542	0.3070	0.3077	0.0085
	2	300	298.8302	99.6100				
	3	300	298.8302	99.6100				
	4	300	299.2009	99.7336				
	5	300	300.6840	100.2280				
	6	300	299.9424	99.9808				

* Mean of Six Observations

TABLE – 27 INTRA DAY AND INTER DAY ANALYSIS OF FORMULATION

(FIRST ORDER DERIVATIVE 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
TPE	1	50	99.3750	99.6642	0.2654	0.2810	0.2670	0.2820
	2	50	99.1340	99.3750				
	3	50	99.6642	99.9373				
		Mean	**99.3910**	**99.6588**				
PCL	1	300	100.3516	99.9808	0.2141	0.2136	0.2137	0.2143
	2	300	99.9808	99.6100				
	3	300	100.3516	99.9808				
		Mean	**100.2280**	**99.8572**				

* Mean of Three Observation

TABLE – 28 RUGGEDNESS STUDY (FIRST ORDER DERIVATIVE METHOD)

Drug	Condition	% Obtained	S.D	%R.S.D	S.E
TPE	Analyst 1	101.0084	0.4641	0.4595	0.0515
	Analyst 2	98.5985	0.4641	0.4707	0.0515
	Instrument 1	99.3911	0.2653	0.2669	0.0294
	Instrument 2	99.6589	0.2810	0.2820	0.0312
PCL	Analyst 1	100.4339	1.8874	1.8793	0.2097
	Analyst 2	101.6698	1.1529	1.1368	0.1281
	Instrument 1	100.2280	1.1529	1.1368	0.1281
	Instrument 2	99.7336	0.2136	0.2141	0.0237

TABLE – 29 RECOVERY ANALYSIS (FIRST ORDER DERIVATIVE METHOD)

Drug	Sample No.	Amount present (µg/ ml)	Amount added (µg/ ml)	Amount estimated* (µg/ ml)	Amount recovered (µg/ ml)	% Recovery*	S.D	% R.S.D	S.E.
TPE	1	2.9690	2.4	5.3702	2.4012	100.05	0.04728	0.0425	0.00525
	2	2.9690	3.0	5.9678	2.9988	99.96			
	3	2.9690	3.6	6.5702	3.6012	100.03			
					Mean	**100.013**			
PCL	1	18.013	14.4	32.378	14.365	99.75	0.4780	0.4779	0.05314
	2	18.013	18	36.121	18.108	100.6			
	3	18.013	21.6	39.569	21.556	99.796			
					Mean	**100.0487**			

* Mean of Three Observation

TABLE - 30 OPTICAL CHARACTERISTICS OF TOLPERISONE HYDROCHLORIDE (RP- HPLC METHOD)

PARAMETERS	TOLPERISONE	PARACETAMOL
Beer's law limit (µg/ml)	2- 10	4-20
Detection wavelength	264nm	264nm
Correlation coefficient (r)	0.998765	0.99976
Regression equation (Y = mx+c)	Y = 78351.5871X+1496.2380	Y = 100917.407X+4939.761905
Slope (m)	78351.5871	100917.407
Intercept (c)	1496.2380	4939.761905
LOD (µg/ml)	0.130196	0.2389
LOQ (µg/ml)	0.3945339	0.723351
Standard error	22004.0421	5807.11282

*****Mean of three observations

TABLE - 31 QUANTIFICATION OF FORMULATION FOR RP - HPLC METHOD

Drug	Sample No.	Labeled amount (mg/ tab)	Amount found (mg/ tab)*	Percentage obtained*	Average (%)	S.D	% R.S.D.	S.E.
TPE	1	150	149.05	99.36	99.83	0.28304	0.28354	0.00786
	2	150	149.90	99.93				
	3	150	149.95	99.96				
	4	150	149.78	99.56				
	5	150	150.01	100.00				
	6	150	150.09	100.06				
PCL	1	325	325.5	100.15	99.935	0.00460	0.00460	0.16574
	2	325	324.3	99.78				
	3	325	325.03	100.01				
	4	325	324.9	99.96				
	5	325	324.05	99.70				
	6	325	325.04	100.01				

TABLE – 32 SYSTEM SUITABILITY PARAMATERS

PARAMETERS	TOLPERISONE HYDROCHLORIDE	PARACETAMOL
Retention time	2.915	4.637
Tailing factor	1.176	1.133
Asymmetrical factor	1.146	1.119
Theoretical plates	5059	7550
Capacity factor	3.21	4.91
Resolution	Between TPE and PCL 9.187	

TABLE - 33 INTRA DAY AND INTER DAY ANALYSIS OF FORMULATION (RP- HPLC 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
TPE	1	150	100.12	98.7	0.4371	0.69291	0.43798	0.69691
	2	150	99.31	99.5				
	3	150	100.0	100.08				
		Mean	**99.6867**	**99.9467**				
PCL	1	325	98.9	99.780	0.72037	0.119304	0.72302	0.11938
	2	325	99.66	99.97				
	3	325	100.34	100.09				
		Mean	**99.633**	**99.933**				

TABLE – 34 RECOVERY ANALYSIS OF FORMULATION (RP - HPLC METHOD)

Drug	Sample No.	Amount present (µg/ ml)	Amount added (µg/ ml)	Amount estimated* (µg/ ml)	Amount recovered (µg/ ml)	% Recovery*	S.D	% R.S.D	S.E.
TPE	1	5.9918	4.8	10.7559	4.7641	99.25	0.24131	0.242488	0.02681
	2	5.9918	6.0	11.9670	5.9752	99.58			
	3	5.9918	7.2	13.1720	7.1802	99.72			
					Mean	**99.51**			
PCL	1	11.990	9.6	21.58	9.59	99.88	0.33866	0.33453	0.03698
	2	11.990	12	23.902	11.912	99.26			
	3	11.990	14.4	26.299	14.309	99.36			
					Mean	**99.50**			

* Mean of Three Observations

TABLE – 35 OPTICAL CHARACTERISTICS (HPTLC METHOD)

PARAMETERS	**TOLPERISONE**	**PARACETAMOL**
Beer's law limit (ng/µl)	20- 100	40-200
Detection wavelength	264nm	264nm
Correlation coefficient (r)	0.999915	0.99976
Regression equation (Y = mx+c)	Y = 9.605714X+0.04761904	Y = 5.586X +3.1809523
Slope (m)	9.605714	5.586
Intercept (c)	0.04761904	3.1809523
LOD (ng/µl)	1.30196	2.098006
LOQ (ng/µl)	3.945339	6.357600
Standard error	5.22666	9.021160

*****Mean of three observations

TABLE – 36 QUANTIFICATION OF FORMULATION - HPTLC METHOD

Drug	**Sample No.**	**Labeled amount (mg/ tab)**	**Amount found (mg/ tab)***	**Percentage obtained***	**Average (%)**	**S.D**	**% R.S.D.**	**S.E.**
TPE	1	150	150.00	100.00	99.883	0.20925	0.20949	0.00581
	2	150	149.45	99.63				
	3	150	149.50	99.66				
	4	150	149.78	99.85				
	5	150	150.25	100.16				
	6	150	150.01	100.00				
PCL	1	325	325.02	100.00	99.978	0.06645	0.06647	0.00184
	2	325	324.9	99.96				
	3	325	324.98	99.99				
	4	325	324.56	99.86				
	5	325	325.01	100.00				
	6	325	325.21	100.06				

TABLE - 37 INTRA DAY AND INTER DAY ANALYSIS OF FORMULATION HPTLC METHO

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
TPE	1	150	99.96	99.30	0.020812	0.69407	0.020822	0.6938
	2	150	100.3	100.12				
	3	150	99.97	100.68				
		Mean	**99.9767**	**99.9467**				
PCL	1	325	99.9	100.09	0.593661	0.2676	0.5957	0.26817
	2	325	100.09	99.56				
	3	325	98.98	99.76				
		Mean	**99.766**	**99.803**				

TABLE - 38 RECOVERY ANALYSIS OF FORMULATION (HPTLC METHOD)

Drug	Sample No.	Amount present (µg/ ml)	Amount added (µg/ ml)	Amount estimated (µg/ ml)	Amount recovered (µg/ ml)	% Recovery *	S.D	% R.S.D	S.E.
TPE	1	6.01	4.8	10.8119	4.801	100.03	0.24131	0.24248	0.02681
	2	6.01	6.0	11.9965	5.9865	99.775			
	3	6.01	7.2	13.2560	7.246	100.63			
					Mean	**99.51**			
PCL	1	12.05	9.6	21.5602	9.5102	99.06	0.47056	0.47285	0.05228
	2	12.05	12	23.9897	11.9397	99.49			
	3	12.05	14.4	26.4508	14.4008	100.00			
					Mean	**99.50**			

* Mean of Three Observations

TABLE - 39 SOLUBILITY PROFILE OF SITAGLIPTIN PHOSPHATE

S.NO	SOLVENT	EXTENT OF SOLUBILITY	CATEGORY
1	Distilled Water	10 mg in more than 10 ml	Insoluble
2	0.1M Sodium Hydroxide	10 mg in 50 µl	Soluble
3	0.1M Hydrochloric acid	10 mg in 30µl	Soluble
4	Methanol	10 mg in 20µl	Soluble
5	Chloroform	10 mg in more than 10 ml	Insoluble
7	Dichloromethane	10 mg in more than 10 ml	Insoluble
8	Ethanol	10 mg in 5 ml	Slightly Soluble
9	Benzene	10 mg in more than 10 ml	Insoluble
10	Glacial Acetic acid	10 mg in 30µl	Soluble
11	Acid Phthalate Buffer(pH 3.0)	10 mg in 50 µl	Soluble
12	Neutralized Phthalate Buffer (pH 5.0)	10 mg in ml 20 µl	Soluble
13	Borate buffer(pH 9.0)	10 mg in 40 µl	Soluble
14	Phosphate buffer(pH 7.0)	10 mg in 10 µl	Soluble
15	Acetone	10 mg in 5 ml	Slightly Soluble
16	Carbon tetra chloride	10 mg in more than 10 ml	Insoluble
17	Dicholro methane	10 mg in more than 10 ml	Insoluble
18	Iso propanol	10 mg in 5 ml	Slightly Soluble
19.	Benzene	10 mg in 1 ml	Sparingly soluble
20.	Diethyl ether	10 mg in 1 ml	Sparingly soluble
21.	Toluene	10 mg in 5 ml	Slightly soluble

TABLE - 40 SOLUBILITY PROFILE OF SIMVASTATIN

S.NO	SOLVENT	EXTENT OF SOLUBILITY	CATEGORY
1	Distilled Water	10 mg in more than 10 ml	Insoluble
2	0.1M Sodium Hydroxide	10 mg in more than 10 ml	Insoluble
3	0.1M Hydrochloric acid	10 mg in more than 10 ml	Insoluble
4	Methanol	10 mg in ml 20 µl	Very Soluble
5	Chloroform	10 mg in 40 µl	Freely Soluble
7	Dichloromethane	10 mg in 10 µl	Soluble
8	Ethanol	10 mg in 60 µl	Soluble
9	Benzene	10 mg in more than 1 ml	Slightly Soluble
10	Glacial Acetic acid	10 mg in 30 µl	Soluble
11	Acid Phthalate Buffer(pH 3.0)	10 mg in more than 10 ml	Insoluble
12	Neutralized Phthalate Buffer (pH5.0)	10 mg in more than 10 ml	Insoluble
13	Borate buffer(pH 9.0)	10 mg in more than 10 ml	Insoluble
14	Phosphate buffer (pH 7.0)	10 mg in 40 µl	Soluble
15	Acetone	10 mg in ml 20 µl	Freely Soluble
16	Carbon tetra chloride	10 mg in 40 µl	Soluble
17	Dicholro methane	10 mg in more than 10 ml	Insoluble
18.	Isopropanol	10 mg in 40 µl	Soluble
19.	Benzene	10 mg in more than 10 ml	Insoluble
20.	Diethyl ether	10 mg of solute in 40 µl	Freely soluble
21	Toluene	10 mg in 6ml	Slightly soluble

TABLE - 41 OPTICAL CHARACTERISTICS - DERIVATIVE SPECTROSCOPY METHOD

PARAMETERS	SITAGLIPTIN PHOSPHATE 277 nm	SIMVASTATIN 238 nm
Beer's law limit (µg/ ml)	10-50	4-20
Molar absorptivity (L mol-1 cm-1)	237.7307	680.16975
Sand ell's sensitivity (µg/cm2/0.001A.U)	2.489933147	0.6616625709
Correlation coefficient (r)	0.999301635	0.999315344
Regression equation (y = mx+c)	Y =0.000401714x+0.000357 48	Y=0.001511429x+0.000252 381
Slope (m)	0.000401714	0.001511429x
Intercept (c)	0.00035748	0.000252381
LOD (µg/ ml)	1.63810574	0.272922
LOQ (µg/ ml)	4.9635679	0.82703683
Standard Error	0.000314188	0.000468178

TABLE - 42 SYNTHETIC MIXTURES (DERIVATIVE SPECTROSCOPY METHOD)

Drug	Sample No.	Concentration (µg/ml)	Amount found (µg/ ml)	Percentage obtained	Average (%)	S.D	% R.S.D.	S.E.
SIM	1	4	4.002	100.05	100.034	0.14631	0.14626	0.00585
	2	8	8.021	100.262				
	3	12	11.984	99.86				
	4	16	15.998	99.98				
	5	20	20.004	100.02				
SITA	1	10	9.99	99.09	99.796	0.43202	0.43290	0.017281
	2	20	20.023	100.115				
	3	30	29.960	99.86				
	4	40	39.899	99.747				
	5	50	50.089	100.17				

TABLE – 43 QUANTIFICATION OF FORMULATION (DERIVATIVE SPECTROSCOPY METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage obtained*	Average (%)	S.D	% R.S.D.	S.E.
SIM	1	40	40.30	100.75	99.97	0.600347	0.600347	0.01667
	2	40	39.96	99.90				
	3	40	40.10	100.25				
	4	40	39.98	99.95				
	5	40	39.57	98.92				
	6	40	40.02	100.05				
SITA	1	100	99.09	99.09	99.745	0.79458	0.79658	0.02207
	2	100	98.51	98.51				
	3	100	99.92	99.92				
	4	100	100.66	100.66				
	5	100	100.23	100.23				
	6	100	100.06	100.06				

TABLE - 44 INTER DAY AND INTRADAY ANALYSIS OF FORMULATION (DERIVATIVE 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
SIM	1	40	99.401	100.686	0.42377	0.695547	0.425211	0.695834
	2	40	99.432	99.300				
	3	40	100.15	99.890				
		Mean	**99.661**	**99.958**				
SITA	1	100	99.3516	100.10	0.4581	0.290478	0.4588	0.29089
	2	100	99.9808	99.876				
	3	100	100.2431	100.453				
		Mean	**99.8585**	**100.143**				

TABLE - 45 RUGGEDNESS STUDY ((DERIVATIVE METHOD)

Drug	Condition	% Obtained	S.D	%R.S.D	S.E
SIM	Analyst 1	99.733	0.5001	0.501354	0.03125
	Analyst 2	100.05	0.014135	0.14142	0.00356
	Instrument 1	100.03	0.014142	0.14132	0.00352
	Instrument 2	100.045	0.00707	0.001768	0.007068
SITA	Analyst 1	99.52	0.799031	0.80284	0.199758
	Analyst 2	99.89	0.4196	0.41766	0.046626
	Instrument 1	100.42	0.220377	0.22076	0.07528
	Instrument 2	99.937	0.0947	0.09448	0.023688

TABLE - 46 RECOVERY ANALYSIS OF FORMULATION (DERIVATIVE METHOD)

Drug	Sample No.	Amount present (µg/ ml)	Amount added (µg/ ml)	Amount estimated (µg/ ml)	Amount recovered (µg/ ml)	% Recovery *	S.D	% R.S.D	S.E.
SIM	1	12.01	9.6	21.634	9.624	100.25	0.52538	0.52395	0.05837
	2	12.01	12	23.982	11.972	99.76			
	3	12.01	14.4	26.527	14.517	100.81			
					Mean	**100.2733**			
SITA	1	30.02	24	53.876	23.856	99.40	0.416773	0.418153	0.04630
	2	30.02	30	59.850	29.84	99.46			
	3	30.02	36	66.074	36.054	100.15			
					Mean	**99.67**			

* Mean of Three Observation

TABLE - 47 OPTICAL PARAMETERS OF SITAGLIPTIN PHOSPHATE AND SIMVASTATIN BY RP-HPLC METHOD

PARAMETERS	SITAGLIPTIN PHOSPHATE 277 nm	SIMVASTATIN 238 nm
Beer's law limit (µg/ ml)	160-240	64-96
Sandell's sensitivity (µg/cm^2/0.001 A.U)	0.00030964	4.73939e-05
Correlation coefficient (r)	0.999330714	0.999944317
Regression equation (y= mx+c)	Y =3.229554643 x +0.4440892	Y=21.09977978x - 2.5279684520
Slope (m)	3.229554643	21.09977978
Intercept (c)	0.4440892	- 2.5279684520
LOD (µg/ ml)	0.003101074	0.001460235
LOQ (µg/ ml)	0.009397194	0.004424953
Standard error	11.407847	8.59299409

TABLE - 48 QUANTIFICATION OF FORMULATION BY RP-HPLC METHOD

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage obtained*	Average (%)	S.D	% R.S.D.	S.E.
SIM	1	40	40.30	100.75	100.108	0.410614	0.410119	0.01140
	2	40	39.80	99.50				
	3	40	39.94	100.25				
	4	40	40.02	100.05				
	5	40	39.98	99.95				
	6	40	40.09	100.225				
SITA	1	100	98.02	98.02	99.583	0.85773	0.861322	0.02382
	2	100	100.54	100.54				
	3	100	99.59	99.59				
	4	100	99.76	99.76				
	5	100	100.11	100.11				
	6	100	99.48	99.48				

TABLE – 49 INTER DAY AND INTRADAY ANALYSIS OF FORMULATION

(RP-HPLC 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
SIM	1	40	100.09	99.87	0.300676	0.459031	0.301085	0.460217
	2	40	99.523	100.12				
	3	40	99.98	99.23				
		Mean	**99.8643**	**99.74**				
SITA	1	100	100.07	99.32	0.56748	0.415833	0.567858	0.417411
	2	100	99.31	99.46				
	3	100	100.42	100.10				
		Mean	**99.933**	**99.62**				

TABLE – 50 RECOVERY ANALYSIS (RP-HPLC METHOD)

Drug	Sample No.	Amount present (µg/ ml)	Amount added (µg/ ml)	Amount estimated (µg/ ml)	Amount recovered (µg/ ml)	% Recovery*	S.D	% R.S.D	S.E.
SITA	1	200.02	160	359.342	159.322	99.57	0.607207	0.60659	0.06747
	2	200.02	200	401.5517	201.531	100.765			
	3	200.02	240	439.99	239.97	99.98			
					Mean	**100.105**			
SIM	1	80.01	64	144.09	64.08	100.12	0.75719	0.075653	0.00841
	2	80.01	80	160.1253	80.11	100.14			
	3	80.01	96	176.102	96.092	100.00			
					Mean	**100.086**			

* Mean of Three Observations

TABLE - 51 SYSTEM SUITABILITY PARAMATERS FOR RP-HPLC METHOD

PARAMETERS	SITAGLIPTIN PHOSPHATE	SIMVASTATIN
Retention time	4.03	6.8
Tailing factor	1.033	1.23
Asymmetrical factor	1.828	1.429
Theoretical plates	3999	10164
Capacity factor	1.03	2.65

TABLE – 52 OPTICAL CHARACTERS – HPTLC METHOD

PARAMETERS	SITAGLIPTIN PHOSPHATE 277 nm	SIMVASTATIN 238 nm
Beer's law limit (µg/ml)	100-500	40-200
Sandell's sensitivity(µg/cm^2/0.001 A.U)	0.000105384	3.60042E-05
Correlation coefficient (r)	0.999721032	0.999725175
Régression équation (y = mx+c)	Y = 9.489082857x+6.015714286	Y = 27.77457143 - 3.423809524
Slope (m)	9.489082857	27.77457143
Intercept (c)	-6.015714286	-3.423809524
LOD (µg/ ml)	1.710676464	0.182625
LOQ (µg/ ml)	5.183680	0.553493308
Standard error	46.89177	54.4915777

TABLE – 53 QUANTIFICATION OF FORMULATION BY HPTLC METHOD

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage obtained*	Average (%)	S.D	% R.S.D.	S.E.
SIM	1	40	40.20	100.5	99.78	0.632712	0.634101	0.01757
	2	40	39.94	99.87				
	3	40	39.62	99.07				
	4	40	40.07	100.07				
	5	40	39.58	98.95				
	6	40	40.09	100.22				
SITA	1	100	100.24	100.245	99.983	0.175351	0.175381	0.004871
	2	100	99.72	99.72				
	3	100	99.99	99.99				
	4	100	100.01	100.01				
	5	100	100.05	100.05				
	6	100	99.88	99.88				

TABLE - 54 INTER AND INTRADAY ANALYSIS OF FORMULATION (HPTLC 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
SIM	1	40	99.65	99.97	0.464901	0.08544	0.464082	0.085474
	2	40	100.53	100.04				
	3	40	100.35	99.87				
		Mean	**100.17**	**99.96**				
SITA	1	100	99.85	99.68	0.241937	0.225389	0.241848	0.225524
	2	100	99.95	100.06				
	3	100	100.31	100.08				
		Mean	**100.0367**	**99.94**				

TABLE - 55 RECOVERY ANALYSIS (HPTLC METHOD)

Drug	Sample No.	Amount present (µg/ ml)	Amount added (µg/ ml)	Amount estimated (µg/ ml)	Amount recovered (µg/ ml)	% Recovery*	S.D	% R.S.D	S.E.
SITA	1	300.01	240	540.113	240.103	99.57	0.22141	0.22182	0.024605
	2	300.01	300	598.675	298.695	99.95			
	3	300.01	360	659.856	359.846	99.957			
					Mean	**99.82**			
SIM	1	120.1	96	215.985	95.88	99.880	0.42461	0.424151	0.04718
	2	120.1	120	239.920	119.82	99.85			
	3	120.1	144	264.996	144.896	100.60			
					Mean	**100.11**			

* Mean of Three Observations

TABLE - 56 SOLUBILITY PROFILE OF KETOPROFEN

S.NO	SOLVENT	EXTENTOF SOLUBILITY	CATEGORY
1	Distilled Water	10 mg in more than 10 ml	Insoluble
2	0.1M Sodium Hydroxide	10 mg in 10ml	Insoluble
3	0.1M Hydrochloric acid	10 mg in 10ml	Insoluble
4	Methanol	10 mg in 20µl	Soluble
5	Chloroform	10 mg in 40µl	Soluble
7	Dichloromethane	10 mg in more than 10 ml	Insoluble
8	Ethanol	10 mg in 30µl	Freely Soluble
9	Benzene	10 mg in more than 10 ml	Insoluble
10	Glacial Acetic acid	10 mg in 30µl	Soluble
11	Acid Phthalate Buffer(pH 3.0)	10 mg in 50 µl	Soluble
12	Neutralized Phthalate Buffer (pH 5.0)	10 mg in 10 ml	Insoluble
13	Borate buffer(pH 9.0)	10 mg in 40 µl	Soluble
14	Phosphate buffer(pH 7.0)	10 mg in 0.5ml	Slightly Soluble
15	Acetone	10 mg in 1.5 ml	Slightly Soluble
16	Carbon tetra chloride	10 mg in 0.9 ml	Practically Insoluble
17	Dicholro methane	10 mg in more than 10 ml	Insoluble
18	Iso propanol	10 mg in 5 ml	Slightly Soluble
19.	Benzene	10 mg in 20µl	Soluble
20.	Diethyl ether	10 mg in 10µl	Soluble
21.	Toluene	10 mg in 5 ml	Slightly soluble

TABLE 57 SOLUBILITY PROFILE OF THIOCOLCHICOSIDE

S.NO	SOLVENT	EXTENT OF SOLUBILITY	CATEGORY
1	Distilled Water	10 mg in 10 µl	Freely soluble
2	0.1M Sodium Hydroxide	10 mg in more than 10 ml	Insoluble
3	0.1M Hydrochloric acid	10 mg in more than 10 ml	Insoluble
4	Methanol	10 mg in ml 20 µl	Very Soluble
5	Chloroform	10 mg in 40 µl	Freely Soluble
7	Dichloromethane	10 mg in 10 µl	Soluble
8	Ethanol	10 mg in 20 µl	Soluble
9	Benzene	10 mg in 0.5 ml	Slightly Soluble
10	Glacial Acetic acid	10 mg in 30 µl	Soluble
11	Acid Phthalate Buffer(pH 3.0)	10 mg in more than 100 ml	Insoluble
12	Neutralized Phthalate Buffer (pH5.0)	10 mg in 2ml	Insoluble
13	Borate buffer(pH 9.0)	10 mg in more than 10 ml	Insoluble
14	Phosphate buffer (pH 7.0)	10 mg in 40 µl	Soluble
15	Acetone	10 mg in ml 20 µl	Freely Soluble
16	Carbon tetra chloride	10 mg in 40 µl	Soluble
17	Dicholro methane	10 mg in more than 10 ml	Insoluble
18.	Isopropanol	10 mg in 20 µl	Soluble
19.	Benzene	10 mg in more than 3 ml	Insoluble
20.	Diethyl ether	10 mg of solute in 40 µl	Freely soluble
21	Toluene	10 mg in 10ml	Practically insoluble

TABLE – 58 OPTICAL CHARACTERISTICS - DERIVATIVE SPECTROSCOPY METHOD

PARAMETERS	THIOCOLCHICOSIDE	KETOPROFEN
Beer's law limit (µg/ml)	4-24	50-300
Molar absorptivity (L mol^{-1} $cm^{-1)}$	237.7307	680.16975
Sand ell's sensitivity(µg/cm^2/0.001 A.U)	1.663201663	0.29243407
Correlation coefficient (r)	0.999590	0.999945
Régression équation (y = mx+c)	Y=0.00060125x+0.0000714286	Y=0.003419571x+0.00009285
Slope (m)	0.00060125	0.003419571
Intercept (c)	0.0000714286	0.00009285
LOD (µg/ml)	0.31288	3.9111
LOQ (µg/ml)	0.948150	11.85187699
Standard error	0.000162876	0.000421906

TABLE – 59 SYNTHETIC MIXTURES - DERIVATIVE SPECTROSCOPY METHOD

Drug	Sample No.	Concentration (µg/ ml)	Amount found (µg/ ml)	Percentage obtained*	Average (%)	S.D	% R.S.D.	S.E.
THI	1	4	3.99	99.75	100.11	0.248551	0.24824	0.00690
	2	8	8.03	100.37				
	3	12	11.99	99.91				
	4	16	16.04	100.25				
	5	20	20.01	100.05				
	6	24	24.08	100.33				
KET	1	50	50.09	100.18	100.085	0.24419	0.24398	0.00678
	2	100	100.51	100.51				
	3	150	149.67	99.78				
	4	200	200.02	100.01				
	5	250	250.05	100.02				
	6	300	300.03	100.01				

TABLE – 60 QUANTIFICATION OF FORMULATION

(DERIVATIVE SPECTROSCOPY METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage obtained*	Average (%)	S.D	% R.S.D.	S.E.
THI	1	4	3.99	99.75	100.00	0.866025	0.866025	0.024056
	2	4	4.01	100.25				
	3	4	4.05	101.25				
	4	4	3.98	99.5				
	5	4	3.95	98.75				
	6	4	4.02	100.5				
KET	1	50	50.09	100.18	100.2633	0.419031	0.41793	0.01164
	2	50	50.51	101.02				
	3	50	49.96	99.92				
	4	50	49.98	99.96				
	5	50	50.02	100.04				
	6	50	50.20	100.46				

TABLE - 61 INTER DAY AND INTRADAY ANALYSIS OF FORMULATION

(DERIVATIVE 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
THI	1	4	100.09	99.6867	0.225019	0.306656	0.224809	0.30668
	2	4	100.32	100.300				
	3	4	99.87	99.990				
		Mean	**100.093**	**99.992**				
KET	1	50	99.745	100.09	0.190922	0.181288	0.191018	0.020143
	2	50	99.9808	99.776				
	3	50	100.123	100.09				
		Mean	**99.9496**	**99.9853**				

TABLE - 62 RUGGEDNESS STUDY (DERIVATIVE METHOD)

Drug	Condition	% Obtained	S.D	%R.S.D	S.E
THI	Instrument 1	98.66	0.8496	0.8611	0.0236
	Analyst 1	99.07	0.8289	0.8367	0.0230
KET	Instrument 2	98.97	1.2756	1.2887	0.0354
	Analyst 2	99.03	0.8143	0.8223	0.0226

TABLE - 63 RECOVERY ANALYSIS OF FORMULATION (DERIVATIVE METHOD)

Drug	Sample No.	Amount present (µg/ ml)	Amount added (µg/ ml)	Amount estimated (µg/ ml)	Amount recovered (µg/ ml)	% Recovery *	S.D	% R.S.D	S.E.
THI	1	3.999	3.2	7.1992	3.2002	100.000	0.340196	0.339066	0.0378
	2	3.999	4	8.0121	4.0131	100.32			
	3	3.999	4.8	8.8321	4.8331	100.68			
					Mean	**100.333**			
KET	1	50.02	40	90.087	40.067	100.167	0.120371	0.12033	0.013375
	2	50.02	50	99. 987	49.967	99.934			
	3	50.02	60	110.02	60.000	100.000			
					Mean	**100.0338**			

* Mean of Three Observations

TABLE - 64 OPTICAL PARAMETERS OF THIOCOLCHICOSIDE AND KETOPROFEN BY RP-HPLC METHOD

PARAMETERS	THIOCOLCHICOSIDE	KETOPROFEN
Beer's law limit (µg/ml)	6.4 - 9.6	80 - 120
Sandell's sensitivity(µg/cm^2/0.001AU)	7.902205E-05	0.000126105
Correlation coefficient (r)	0.999626537	0.99979536
Régression équation (y = mx+c)	Y=12.65494x+0.253053571	Y=7.92987857x+2.429785714
Slope (m)	12.65494	7.92987857
Intercept (c)	0.253053571	2.429785714
LOD (µg/ml)	2.167946897	27.098527
LOQ (µg/ml)	6.569536053	82.11674919
Standard error	1.336900629	7.750725574

TABLE – 65 QUANTIFICATION OF FORMULATION (BY RP-HPLC METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage obtained*	Average (%)	S.D	% R.S.D.	S.E.
THI	1	4	4.03	100.75	100.1333	0.621825	0.620997	0.017273
	2	4	3.98	99.50				
	3	4	4.06	101.05				
	4	4	3.99	99.75				
	5	4	4.00	100.0				
	6	4	3.99	99.75				
KET	1	50	49.92	99.84	100.1933	0.554929	0.553859	0.015415
	2	50	50.54	100.54				
	3	50	50.10	101.08				
	4	50	49.76	99.52				
	5	50	50.11	100.22				
	6	50	49.48	99.96				

TABLE - 66 INTER DAY AND INTRADAY ANALYSIS OF FORMULATION (RP-HPLC 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
THI	1	4	99.78	99.97	0.152753	0.137961	0.152885	0.13786
	2	4	100.08	100.02				
	3	4	99.88	100.23				
		Mean	**99.9133**	**100.073**				
KET	1	50	100.04	99.56	0.142244	0.272213	0.142282	0.272567
	2	50	99.81	99.98				
	3	50	100.07	100.07				
		Mean	**99.9733**	**99.87**				

TABLE - 67 RECOVERY ANALYSIS (RP-HPLC METHOD)

Drug	Sample No.	Amount present (µg/ ml)	Amount added (µg/ ml)	Amount estimated * (µg/ ml)	Amount recovered (µg/ ml)	% Recovery *	S.D	% R.S.D	S.E.
THI	1	7.999	6.4	14.435	6.436	100.565	0.526996	0.524635	0.05855
	2	7.999	8	15.989	7.99	99.875			
	3	7.999	9.6	17.687	9.688	100.91			
					Mean	**100.45**			
KET	1	100.02	80	180.132	80.112	100.14	0.111369	0.111264	0.012374
	2	100.02	100	199.987	99.967	99.967			
	3	100.02	120	220.231	120.211	100.175			
					Mean	**100.094**			

* Mean of Three Observation

TABLE – 68 SYSTEM SUITABILITY PARAMATERS FOR RP-HPLC METHOD

PARAMETERS	THIOCOLCHICOSIDE	KETOPROFEN
Retention time	3.743	7.903
Tailing factor	1.23	1.75
Asymmetrical factor	1.51	1.33
Theoretical plates	8308	11974
Capacity factor	1.10	1.26

TABLE- 69 SOLUBILITY PROFILE OF DESLORATADINE

S.No.	SOLVENTS	EXTENT OF SOLUBILITY	CATEGORY
1	Distilled Water	10 mg in more than 6 ml	Very slightly soluble
2	0.1M Sodium Hydroxide	10 mg in 3ml	Sparingly Soluble
3	0.1M Hydrochloric acid	10 mg in 6ml	Very slightly soluble
4	Methanol	10 mg in 20µl	Soluble
5	Chloroform	10 mg in more than 10 ml	Insoluble
7	Dichloromethane	10 mg in more than 10 ml	Insoluble
8	Ethanol	10 mg in 10 µl	Freely Soluble
9	Benzene	10 mg in more than 10 ml	Insoluble
10	Glacial Acetic acid	10 mg in 30µl	Soluble
11	Acid Phthalate Buffer(pH 3.0)	10 mg 7.5ml	Insoluble
12	Neutralized Phthalate Buffer (pH 5.0)	10 mg in 10ml	Insoluble
13	Borate buffer(pH 9.0)	10 mg in 8ml	Insoluble
14	Phosphate buffer(pH 7.0)	10 mg in 10 ml	Insoluble
15	Acetone	10 mg in 5 ml	In soluble
16	Carbon tetra chloride	10 mg in more than 10 ml	Insoluble
17	Dicholro methane	10 mg in more than 10 ml	Insoluble
18	Iso propanol	10 mg in 10 µl	Soluble
19	Diethyl ether	10 mg in 1 ml	Sparingly soluble
20	Toluene	10 mg in 5 ml	Slightly soluble

TABLE- 70 SOLUBILITY PROFILE OF AMBROXOL HYDROCHLORIDE

S.No.	SOLVENTS	EXTENT OF SOLUBILITY	CATEGORY
1	Distilled water	10 mg in 600µl	Sparingly soluble
2	0.1M Hydrochloric acid	10 mg in 1 ml	Sparingly soluble
3	0.1M Sodium Hydroxide	10 mg in more than 10 ml	In soluble
4	Methanol	10 mg in 80µl	Freely soluble
5	Ethanol	10 mg in 500µl	Sparingly soluble
6	Chloroform	10 mg in more than 10 ml	In soluble
7	DMF	10 mg in 60µl	Freely soluble
8	Acetone	10 mg in more than 10 ml	Insoluble
9	Toluene	10 mg in more than 10 ml	Insoluble
10	n – Butanol	10 mg in 8 ml	Slightly Soluble
11	Acetonitrile	10 mg in more than 10 ml	Insoluble
12	n-Hexane	10 mg in more than 10 ml	Insoluble
13	Isopropyl alcohol	10 mg in 5 ml	Slightly soluble
14	Ethyl acetate	10 mg in more than 10 ml	In soluble
15	10% Glacial acetic acid	10 mg in 1 ml	Sparingly soluble
16	Phthalate buffer (pH 3.0)	10 mg in 1 ml	Sparingly soluble
17	Phthalate buffer (pH 5.0)	10 mg in 5 ml	Slightly soluble
18	Phosphate buffer (pH 7.0)	10 mg in 7 ml	Slightly soluble
19	Borate buffer (pH 9.0)	10 mg in 7 ml	Slightly soluble
20	Benzene	10 mg in more than 10 ml	Insoluble
21	Dichloro methane	10 mg in more than 10 ml	In soluble

TABLE- 71 OPTICAL CHARACTERISTICS OF DESLORATADINE (ABSORPTION RATIO METHOD)

PARAMETERS	DESLORATADINE at 244nm	DESLORATADINE at 288nm
Beer's law limit (µg/ ml)	0.5-2.5	0.5-2.5
Molar absorptivity(l mol^{-1} $cm^{-1)}$	237.7307	680.16975
Sandell's sensitivity (µg/cm^2/0.001 A.U)	0.009400516	0.00550635
Correlation coefficient (r)	0.9996279	0.99992637
Regression équation (y = mx+c)	Y=0.106377143x+0.0011 7142	Y=0.181605714x+0.0000 904
Slope (m)	0.106377143	0.181605714
Intercept (c)	0.001171429	0.00009047
LOD (µg/ ml)	0.005467376	0.012970
LOQ (µg/ ml)	0.016567805	0.0393060
Standard error	0.0003035528	0.002304788

TABLE- 72 OPTICAL CHARACTERISTICS OF AMBROXOL HYDROCHLORIDE (ABSORPTION RATIO METHOD)

PARAMETERS	AMBROXOL at 244nm	AMBROXOL AT 288 nm
Beer's law limit (µg/ml)	8 - 40	8 - 40
Molar absorptivity (L mol^{-1} $cm^{-1)}$	8581.4656	11034.68
Sand ell's sensitivity (µg/cm^2/0.001A.U)	0.059836731	0.412918449
Correlation coefficient (r)	0.9997136	0.999726338
Régression équation (Y = mx+c)	Y= 0.01671214x - 0.0015238	Y= 0.002421786x - 0.000452381
Slope (m)	0.01671214	0.002421786x
Intercept (c)	- 0.0015238	0.000452381
LOD (µg/ml)	0.171113	0.0.349338
LOQ (µg/ml)	0.51852638	1.05860
Standard error	0.006693433	0.000942857

TABLE – 73 SYNTHETIC MIXTURES (ABSORPTION RATIO METHOD)

Drug	Sample No.	Concentration (µg/ ml)	Amount found (µg/ ml)	Percentage obtained*	Average (%)	S.D	% R.S.D.	S.E.
DES	1	0.5	0.5005	100.1	100.022	0.51178	0.5116	0.02047
	2	1	1.007	100.7				
	3	1.5	1.489	99.26				
	4	2	2.001	100.05				
	5	2.5	2.500	100.0				
AMB	1	8	8.03	100.37	100.238	0.23381	0.23326	0.009353
	2	16	16.09	100.56				
	3	24	24.02	100.08				
	4	32	32.07	100.21				
	5	40	39.99	99.97				

TABLE – 74 QUANTIFICATION OF FORMULATION (ABSORPTION RATIO METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage obtained*	Average (%)	S.D	% R.S.D.	S.E.
DES	1	5	5.03	100.6	100.2183	0.541125	0.539946	0.015031
	2	5	4.98	99.60				
	3	5	4.99	99.8				
	4	5	5.02	100.4				
	5	5	5.05	101.01				
	6	5	4.95	99.90				
AMB	1	75	74.97	99.96	99.97	0.31686	0.316955	0.008802
	2	75	74.76	99.68				
	3	75	75.34	100.45				
	4	75	74.68	99.57				
	5	75	75.03	100.04				
	6	75	75.09	100.12				

TABLE - 75 INTER DAY AND INTRADAY ANALYSIS OF FORMULATION

(ABSORPTION RATIO 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
DES	1	5	99.98	99.786				
	2	5	99.68	100.300	0.212211	0.552436	0.212388	0.550644
	3	5	100.09	100.89				
		Mean	**99.9167**	**100.3253**				
AMB	1	75	100.021	99.54				
	2	75	99.88	99.876	0096645	0.335	0.096656	0.335419
	3	75	100.065	100.21				
		Mean	**99.98**	**99.87**				

TABLE -76 RUGGEDNESS STUDY (ABSORPTION RATIO METHOD)

Drug	Condition	% Obtained	S.D	%R.S.D	S.E
DES	Analyst 1	99.733	0.5001	0.501354	0.03125
	Analyst 2	100.05	0.014135	0.14142	0.00356
	Instrument 1	100.03	0.014142	0.14132	0.00352
	Instrument 2	100.045	0.00707	0.001768	0.007068
AMB	Analyst 1	99.52	0.799031	0.80284	0.199758
	Analyst 2	99.89	0.4196	0.41766	0.046626
	Instrument 1	100.42	0.220377	0.22076	0.07528
	Instrument 2	99.937	0.0947	0.09448	0.023688

TABLE - 77 RECOVERY ANALYSIS OF FORMULATION (ABSORPTION RATIO METHO)

Drug	Sample No.	Amount present (µg/ ml)	Amount added (µg/ ml)	Amount estimated* (µg/ ml)	Amount recovered (µg/ ml)	% Recovery*	S.D	% R.S.D	S.E.
DES	1	1.002	0.8	1.8135	0.8115	101.4375	0.616504	0.610224	0.0685
	2	1.002	1	2.0052	1.0032	100.32			
	3	1.002	1.2	2.218	1.216	101.33			
					Mean	**101.0292**			
AMB	1	15.032	12	27.1214	12.0894	100.745	0.521063	0.51987	0.05789
	2	15.032	15	30.0678	15.0358	100.238			
	3	15.032	18	32.9786	17.9466	99.703			
					Mean	**100.2287**			

* Mean of Three Observation

TABLE 78 OPTICAL PARAMETERS OF DESLORATADINE AND AMBROXOL HYDROCHLORIDE (DERIVATIVE METHOD)

PARAMETERS	DESLORATADINE at 277nm	AMBROXOL at 320nm
Beer's law limit (µg/ml)	5-25	75-375
Sandell's sensitivity(µg/cm^2/0.001 A.U)	1.350308642	2.8162214
Correlation coefficient (r)	0.999219	0.9999834
Regression equation (y = mx+c)	Y=0.000740571x -.000051714)	Y=0.000355086x -0.000204762
Slope (m)	0.000740571x	0.000355086x
Intercept (c)	-000051714	-0.000204762
LOD (µg/ml)	0.1446132	50.141566
LOQ (µg/ml)	0.43762523	151.94414
Standard error	0.000306128	0.000320565

TABLE – 79 SYNTHETIC MIXTURES (DERIVATIVE METHOD)

Drug	Sample No.	Concentration (µg/ ml)	Amount found (µg/ ml)	Percentage obtained*	Average (%)	S.D	% R.S.D.	S.E.
DES	1	5	4.987	100.8				
	2	10	9.998	99.6				
	3	15	15.09	100.6	100.52	0.59329	0.59022	0.02347
	4	20	19.98	101.2				
	5	25	24.89	100.4				
AMB	1	75	75.09	100.12				
	2	150	149.07	99.38				
	3	225	225.12	100.06	99.914	0.302291	0.30255	0.01209
	4	300	300.04	100.01				
	5	375	375.03	100.00				

TABLE – 80 QUANTIFICATION OF FORMULATION (DERIVATIVE METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage obtained*	Average (%)	S.D	% R.S.D.	S.E.
DES	1	5	5.04	100.8				
	2	5	4.98	99.6				
	3	5	5.03	100.6				
	4	5	5.06	101.2	100.5	0.532917	0.530265	0.0148
	5	5	5.02	100.4				
	6	5	5.02	100.4				
AMB	1	75	75.47	100.62				
	2	75	75.69	100.93				
	3	75	74.99	99.99				
	4	75	75.13	100.17	99.82	1.0461	1.0479	0.0290
	5	75	74.34	99.12				
	6	75	73.64	98.1				

TABLE - 81 INTER DAY AND INTRADAY ANALYSIS OF FORMULATION (DERIVATIVE 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
DES	1	5	99.34	100.43	0.361156	0.50816	0.362073	0.50742
	2	5	100.03	100.45				
	3	5	99.87	99.56				
Mean			**99.74**	**100.146**				
AMB	1	75	100.09	99.76	0.55518	0.16802	0.55459	0.16812
	2	75	99.56	99.98				
	3	75	100.67	100.09				
		Mean	**100.106**	**99.943**				

TABLE - 82 RUGGEDNESS STUDY (DERIVATIVE METHOD)

Drug	Condition	Percentage Obtained	SD	%RSD	SE
DES	Analyst 1	99.00	1.5795	1.5954	0.0438
	Analyst 2	99.53	1.8311	1.8396	0.0509
	Instrument 1	99.40	0.6644	0.6684	0.0184
	Instrument 2	99.01	0.4419	0.4463	0.0128
AMB	Analyst 1	99.95	1.7249	1.7256	0.4791
	Analyst 2	99.99	1.8625	1.8602	0.0517
	Instrument 1	101.11	2.0040	1.9820	0.0557
	Instrument 2	99.80	1.0762	1.0783	0.0299

TABLE – 83 RECOVERY ANALYSIS (DERIVATIVE METHOD)

Drug	Sample No.	Amount present (µg/ ml)	Amount added (µg/ ml)	Amount estimated* (µg/ ml)	Amount recovered (µg/ ml)	% Recovery*	S.D	% R.S.D	S.E.
DES	1	5.07	4	9.0123	3.9423	98.557	1.0661	1.07234	0.11851
	2	5.07	5	10.102	5.032	100.64			
	3	5.07	6	11.022	5.952	99.20			
					Mean	**99.4656**			
AMB	1	74.98	60	135.05	60.07	100.11	0.19	0.190095	0.02111
	2	74.98	75	149.98	75.00	100.00			
	3	74.98	90	164.75	89.77	99.74			
					Mean	**99.95**			

* Mean of Three Observations

TABLE – 84 SOLUBILITY PROFILE OF DOXOFYLLINE

S.No.	SOLVENTS	EXTENT OF SOLUBILITY	CATEGORY
1	Distilled water	10 mg in 10µl	Freely soluble
2	0.1M Hydrochloric acid	10 mg in 20µl	Freely soluble
3	0.1M Sodium Hydroxide	10 mg in 5ml	Sparingly soluble
4	Methanol	10 mg in 40µl	Freely soluble
5	Ethanol	10 mg in 300µl	Sparingly soluble
6	Chloroform	10 mg in more than 10 ml	In soluble
7	DMF	10 mg in 60µl	Freely soluble
8	Acetone	10 mg in 10 µl	Freely soluble
9	Toluene	10 mg in more than 5 ml	Sparingly soluble
10	n – Butanol	10 mg in 5 ml	Slightly Soluble
11	Acetonitrile	10 mg in more than 10 ml	Insoluble
12	n-Hexane	10 mg in more than 10 ml	Insoluble
13	Isopropyl alcohol	10 mg in 5 ml	Slightly soluble
14	Ethyl acetate	10 mg in more than 6ml	Sparingly soluble
15	10% Glacial acetic acid	10 mg in 10 ml	In soluble
16	Phthalate buffer (pH 3.0)	10 mg in 1 ml	Sparingly soluble
17	Phthalate buffer (pH 5.0)	10 mg in 5 ml	Slightly soluble
18	Phosphate buffer (pH 7.0)	10 mg in 6 ml	Slightly soluble
19	Borate buffer (pH 9.0)	10 mg in 10µl	Freely soluble
20	Benzene	10 mg in more than 10 ml	Insoluble
21	Dichloro methane	10 mg in more than 10 ml	In soluble

TABLE – 85 SOLUBILITY PROFILE OF SALBUTAMOL SULPHATE

S.No.	SOLVENTS	EXTENT OF SOLUBILITY	CATEGORY
1	Distilled water	10 mg in 600µl	Sparingly soluble
2	0.1M Hydrochloric acid	10 mg in 1 ml	Sparingly soluble
3	0.1M Sodium Hydroxide	10 mg in more than 10 ml	Insoluble
4	Methanol	10 mg in 80µl	Freely soluble
5	Ethanol	10 mg in 500µl	Sparingly soluble
6	Chloroform	10 mg in more than 10 ml	In soluble
7	DMF	10 mg in 60µl	Freely soluble
8	Acetone	10 mg in more than 10 ml	Insoluble
9	Toluene	10 mg in more than 10 ml	Insoluble
10	n – Butanol	10 mg in 8 ml	Slightly Soluble
11	Acetonitrile	10 mg in more than 10 ml	Insoluble
12	n-Hexane	10 mg in more than 10 ml	Insoluble
13	Isopropyl alcohol	10 mg in 5 ml	Slightly soluble
14	Ethyl acetate	10 mg in more than 10 ml	In soluble
15	10% Glacial acetic acid	10 mg in 1 ml	Sparingly soluble
16	Phthalate buffer (pH 3.0)	10 mg in 1 ml	Sparingly soluble
17	Phthalate buffer (pH 5.0)	10 mg in 5 ml	Slightly soluble
18	Phosphate buffer (pH 7.0)	10 mg in 7 ml	Slightly soluble
19	Borate buffer (pH 9.0)	10 mg in 7 ml	Slightly soluble
20	Benzene	10 mg in more than 10 ml	Insoluble
21	Dichloro methane	10 mg in more than 10 ml	Insoluble

TABLE – 86 OPTICAL CHARACTERISTICS OF DOXOFYLLINE

(SIMULTANEOUS EQUATION METHOD)

PARAMETERS	AT 224 nm	AT 274 nm
Beer's law limit (µg/ml)	5 - 25	5 - 25
Molar absorptivity (L mol^{-1} $cm^{-1)}$	7774.792	9770.672
Sand ell's sensitivity(µg/cm^2/0.001 A.U)	0.034920382	0.027735075
Correlation coefficient (r)	0.999886	0.9999143
Régression équation (Y = mx+c)	Y = 0.0286365x + 0.001242	Y = 0.0360554x - 0.00010952
Slope (m)	0. 0.0286365x	0. 0.0360554x
Intercept (c)	0.001242	-0.00010952
LOD (µg/ml)	0..047535	0.003558
LOQ (µg/ml)	0.144046	0.010789
Standard error	0.004513725	0.0049361

TABLE – 87 OPTICAL CHARACTERISTICS OF SALBUTAMOL SULPHATE

(SIMULTANEOUS EQUATION METHOD)

PARAMETERS	AT 224 nm	AT 274 nm
Beer's law limit (µg/ ml)	5 - 25	5 - 25
Molar absorptivity (L mol^{-1} $cm^{-1)}$	6705.49422	165.60321
Sand ell's sensitivity(µg/cm^2/0.001 A.U)	0.03702058	0.144628099
Correlation coefficient (r)	0.99992957	0.9996607
Régression équation (Y = mx+c)	Y = 0.027012x + 0.0038333	Y = 0.0069142x + (-0.00019523)
Slope (m)	0 .027012	0.0069142x
Intercept (c)	0.0038333	-0.00010952
LOD (µg/ ml)	0.009498	0.000927
LOQ (µg/ ml)	0.287871	0.002811
Standard error	0.0033528	0.0005956

TABLE – 88 SYNTHETIC MIXTURES (SIMULTANEOUS EQUATION METHOD)

Drug	Sample No.	Concentration (µg/ ml)	Amount found (µg/ ml)	Percentage obtained*	Average (%)	S.D	% R.S.D.	S.E.
DOX	1	5	4.96	99.20	99.884	0.386626	0.387075	0.015465
	2	10	10.04	100.04				
	3	15	14.998	99.98				
	4	20	20.012	100.06				
	5	25	25.035	100.14				
SAL	1	5	5.001	100.02	100.106	0.227112	0.226872	0.009084
	2	10	9.986	99.86				
	3	15	15.00	100.00				
	4	20	20.09	100.45				
	5	25	25.05	100.20				

TABLE – 89 QUANTIFICATION FOR FORMULATION

(SIMULTANEOUS EQUATION METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)	Percentage obtained	Average (%)	S.D	% R.S.D.	S.E.
DOX	1	400	399.99	99.99	99.9685	0.178878	0.178946	0.004969
	2	400	400.08	100.02				
	3	400	398.55	99.63				
	4	400	399.71	99.92				
	5	400	400.32	100.08				
	6	400	400.54	100.135				
SAL	1	4	3.99	99.75	100.0	1.118034	1.118034	0.031056
	2	4	4.07	101.75				
	3	4	3.98	99.5				
	4	4	4.03	100.75				
	5	4	3.94	98.50				
	6	4	3.99	99.75				

TABLE - 90 INTER DAY AND INTRADAY ANALYSIS OF FORMULATION (SIMULTANEOUS EQUATION 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
DOX	1	400	100.43	99.98	0.426732	0.434051	0.426135	0.432925
	2	400	99.65	100.04				
	3	400	100.34	100.76				
Mean			**100.14**	**100.26**				
SAL	1	4	99.41	98.98	0.349333	0.400791	0.349896	0.044532
	2	4	100.02	99.53				
	3	4	100.01	99.76				
Mean			**99.81**	**99.42**				

TABLE - 91 RUGGEDNESS STUDY (SIMULTANEOUS EQUATION METHOD)

Drug	Condition	% Obtained	S.D	%R.S.D	S.E
DOX	Analyst 1	101.94	0.4419	0.4463	0.0128
	Analyst 2	99.40	0.6644	0.6684	0.0184
	Instrument 1	101.14	1.8824	1.8801	0.0522
	Instrument 2	99.96	1.5147	1.5153	0.0420
SAL	Analyst 1	99.00	1.5795	1.5954	0.0438
	Analyst 2	99.53	1.8311	1.8396	0.0509
	Instrument 1	99.40	0.6644	0.6684	0.0184
	Instrument 2	101.94	0.2943	0.2887	0.0081

TABLE - 92 RECOVERY ANALYSIS OF FORMULATION

(SIMULTANEOUS EQUATION METHOD)

Drug	Sample No.	Amount present (µg/ ml)	Amount added (µg/ ml)	Amount estimated (µg/ ml)	Amount recovered (µg/ ml)	% Recovery*	S.D	% R.S.D	S.E.
DOX	1	15.002	12	27.0135	12.0115	100.09	0.11898	0.119007	0.01322
	2	15.002	15	29.980	14.978	99.853			
	3	15.002	18	33.0012	17.9992	99.99			
					Mean	**99.9776**			
SAL	1	15.032	12	26.9980	11.966	99.71	0.118749	0.118939	0.013194
	2	15.032	15	30.0234	14.9914	99.942			
	3	15.032	18	33.0100	17.978	99.87			
					Mean	**99.840**			

* Mean of Three Observation

TABLE – 93 OPTICAL CHARACTERISTICS OF DOXOFYLLINE

(AREA UNDER CURVE METHOD)

PARAMETERS	AT 220-230 nm	AT 270-280nm
Beer's law limit (µg/ml)	5 - 25	5 - 25
Molar absorptivity (L mol^{-1} $cm^{-1)}$	774177.56	904911.236
Sand ell's sensitivity(µg/cm^2/0.001 A.U)	0.00360215	0.002894725
Correlation coefficient (r)	0.999767	0.999749
Régression équation (Y = mx+c)	Y = 0.2775836x + 0.01330095	Y= 0.345456x - 0.01396667
Slope (m)	0.2775836	0. 345456
Intercept (c)	0.01330095	- 0.01396667
LOD (µg/ml)	0.00036978	0.00182006
LOQ (µg/ml)	0.001120	0.005515
Standard error	0.0626312	0.0809135

TABLE- 94 OPTICAL CHARACTERISTICS OF SALBUTAMOL SULPHATE (AREA UNDER CURVE METHOD)

PARAMETERS	AT 220-230 nm	AT 270-280nm
Beer's law limit (µg/ml)	5 - 25	5 - 25
Molar absorptivity L mol^{-1} cm^{-1})	64221.4996	14430.4533
Sand ell's sensitivity(µg/cm^2/0.001 A.U)	0.00400035	0.01707034
Correlation coefficient (r)	0.9997400	0.999383
Régression équation (Y = mx+c)	Y = 0.24997714x + 0.052828	Y= 0. 0585811x -- 0.0134857)
Slope (m)	0. 24997714	0. 0585811
Intercept (c)	0.052828	-0.0134857
LOD (µg/ml)	0.002823352	0.00306650
LOQ (µg/ml)	0.0085561	0.0092924
Standard error	0.059622	0.0215303

TABLE – 95 SYNTHETIC MIXTURES (AREA UNDER CURVE METHOD)

Drug	Sample No.	Concentration (µg/ ml)	Amount found (µg/ ml)	Percentage obtained	Average (%)	S.D	% R.S.D.	S.E.
DOX	1 2 3 4 5	5 10 15 20 25	5.001 10.13 14.99 20.09 24.99	100.02 101.3 99.93 100.45 99.96	100.332	0.58057	0.57865	0.023223
SAL	1 2 3 4 5	5 10 15 20 25	4.99 10.00 15.02 20.05 24.98	99.8 100.0 100.13 100.25 99.92	100.02	0.175926	0.17589	0.00703

TABLE – 96 QUANTIFICATION OF FORMULATION

(AREA UNDER CURVE METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)	Percentage obtained	Average (%)	S.D	% R.S.D.	S.E.
DOX	1	400	400.01	100.00	99.997	0.068148	0.06815	0.001893
	2	400	400.04	100.01				
	3	400	399.75	99.93				
	4	400	399.69	99.92				
	5	400	400.09	100.02				
	6	400	400.43	100.107				
SAL	1	4	4.04	101.0	99.833	0.95742	0.959025	0.026595
	2	4	4.03	100.75				
	3	4	3.97	99.25				
	4	4	4.01	100.25				
	5	4	3.95	98.75				
	6	4	3.96	99.00				

TABLE - 97 INTER DAY AND INTRADAY ANALYSIS OF FORMULATION (AREA UNDER 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
D0X	1	400	100.56	100.87	0.301386	0.47184	0.300654	0.470304
	2	400	99.96	100.02				
	3	400	100.21	100.09				
Mean			**100.2433**	**100.3267**				
SAL	1	4	100.32	100.57	0.20232	0.515008	0.202145	0.514974
	2	4	99.98	99.89				
	3	4	99.96	99.56				
Mean			**100.0867**	**100.006**				

TABLE – 98 RUGGEDNESS STUDY (AREA UNDER CURVE METHOD)

Drug	Condition	% Obtained	S.D	%R.S.D	S.E
DOX	Analyst 1 Analyst 2	99.62 99.48	0.806236 0.33709	0.809298 0.338835	0.022395 0.009364
	Instrument 1 Instrument 2	99.76 99.19	0.676668 0.840206	0.678296 0.847039	0.018796 0.023339
SAL	Analyst 1 Analyst 2	99.60 99.71	0.439712 0.495718	0.441463 0.497119	0.012214 0.01377
	Instrument 1 Instrument 2	99.49 99.09	0.904819 0.673402	0.909442 0.679552	0.025134 0.018706

TABLE - 99 RECOVERY ANALYSIS OF FORMULATION (AREA UNDER CURVE METHOD)

Drug	Sample No.	Amount present (µg/ ml)	Amount added (µg/ ml)	Amount estimated* (µg/ ml)	Amount recovered (µg/ ml)	% Recovery *	S.D	% R.S.D	S.E.
DOX	1	15.002	12	27.102	12.100	100.83	0.446132	0.443986	0.04957
	2	15.002	15	30.098	15.096	100.64			
	3	15.002	18	32.999	17.997	99.98			
					Mean	100.483			
SAL	1	15.032	12	27.009	11.977	99.80	0.331385	0.33146	0.036821
	2	15.032	15	29.998	14.966	99.773			
	3	15.032	18	33.098	18.066	100.36			
					Mean	99.977			

* Mean of Three Observations

TABLE –100 OPTICAL CHARACTERISTICS 0F DOXOFYLLINE & SALBUTAMOL SULPHATE (DERIVATIVE METHOD)

PARAMETERS	DOX AT 233 nm	SAL AT 229 nm
Beer's law limit (µg/ ml)	10 - 60	10 - 60
Sand ell's sensitivity (µg/cm^2/0.001A.U)	1.1836	1.30597
Correlation coefficient (r)	0.9998851	0.999794
Régression équation (Y = mx+c)	Y = 0.00084485x + 0.00010476	Y = 0. 000765714x + 0.0000285
Slope (m)	0. 0.00084485	0. 000765714
Intercept (c)	0.00010476	(0.0000285)
LOD (µg/ ml)	0.9930918	0.008375
LOQ (µg/ ml)	3.00936	0.02537
Standard error	0.00026788	0.0001625

TABLE – 101 SYNTHETIC MIXTURES (DERIVATIVE METHOD)

Drug	Sample No.	Concentration (µg/ ml)	Amount found (µg/ ml)	Percentage obtained*	Average (%)	S.D	% R.S.D.	S.E.
DOX	1	10	10.007	100.07	99.912	0.48058	0.48100	0.01334
	2	20	19.78	98.945				
	3	30	30.03	100.1				
	4	40	40.08	100.2				
	5	50	49.99	99.98				
	6	60	60.11	100.18				
SAL	1	10	10.09	100.9	100.15	0.43072	0.430007	0.01196
	2	20	20.03	100.15				
	3	30	29.89	99.63				
	4	40	40.12	100.3				
	5	50	49.98	99.96				
	6	60	59.98	99.96				

TABLE – 102 QUANTIFICATION FOR FORMULATION (DERIVATIVE METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage obtained*	Average (%)	S.D	% R.S.D.	S.E.
DOX	1 2 3 4 5 6	400 400 400 400 400 400	399.56 399.65 400.54 400.76 400.21 398.99	99.89 99.91 100.135 100.19 100.05 99.74	99.9858	0.169364	0.169388	0.004705
SAL	1 2 3 4 5 6	4 4 4 4 4 4	3.98 4.04 4.01 3.98 3.95 3.97	99.5 101.0 100.25 99.5 98.75 99.25	99.70833	0.79713	0.799462	0.022143

TABLE – 103 INTER DAY AND INTRADAY ANALYSIS OF FORMULATION (DERIVATIVE 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
DOX	1	400	99.65	99.76	0.892501	0.1253	0.887693	0.12545
	2	400	101.435	100.01				
	3	400	100.54	99.87				
Mean			**100.5417**	**99.88**				
SAL	1	4	99.84	99.67	0.1789	0.325013	0.179188	0.3261
	2	4	99.73	99.99				
	3	4	100.08	99.34				
Mean			**99.883**	**99.666**				

TABLE – 104 RUGGEDNESS STUDY (DERIVATIVE METHOD)

Drug	Condition	Average*% Obtained	S.D	% R.S.D	S.E.
	Analyst 1	98.37	0.847703	0.861749	0.023547
	Analyst 2	98.42	0.424001	0.430771	0.011778
DOX	Instrument 1	98.67	0.380443	0.385564	0.010568
	Instrument 2	99.92	0.500586	0.507317	0.013905
	Analyst 1	100.61	1.622809	1.61305	0.045078
	Analyst 2	99.29	1.356078	1.365729	0.037669
SAL	Instrument 1	98.71	1.55078	1.575347	0.043197
	Instrument 2	99.11	1.00556	1.014538	0.027932

TABLE – 105 RECOVERY ANALYSIS OF FORMULATION (DERIVATIVE METHOD)

Drug	Sample No.	Amount present (µg/ ml)	Amount added (µg/ ml)	Amount estimated* (µg/ ml)	Amount recovered (µg/ ml)	% Recovery *	S.D	% R.S.D	S.E.
DOX	1	15.002	12	27.004	12.002	100.01	0.12741	0.127317	0.014157
	2		15	29.987	14.985	99.99			
	3	15.002	18	33.042	18.04	100.22			
		15.002							
					Mean	**100.0733**			
SAL	1	15.032	12	26.987	11.955	99.80	0.01914	0.1917	0.002127
	2	15.032	15	29.897	14.865	99.773			
	3	15.032	18	32.998	17.966	99.81			
					Mean	**99.7943**			

* Mean of Three Observations

Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 1, 2013

Research Article

SIMULTANEOUS DETERMINATION OF TOLPERISONE AND PARACETAMOL IN PURE AND FIXED DOSE COMBINATION BY UV – SPECTROPHOTOMETRY

G.ABIRAMI* T.VETRICHELVAN[1]

Departmaent of Pharmaceutical Analysis, Adhiparasakthi College of Pharmacy, Melmaruvathur. Email:abiramiganesan78@gmail.com

Received: 05 Jan 2013, Revised and Accepted: 12 Feb 2013

ABSTRACT

Two new simple accurate and sensitive UV– spectrophotometeric methods have been developed for determination Simultaneous equation and Derivative spectroscopy method for Tolperisone and Paracetamol in bulk and in combined dosage form. Double distilled was used as a solvent. The wavelength selected for Simultaneous method for Tolperisone at 261nm and Paracetamol at 243nm respectively. Beer's law was obeyed with the concentration ranges from 0-2.5µg/ml, 3- 9µg/ml respectively. For derivative spectroscopy method(1-5µg/ml,6-30µg/ml) the zero order spectrum was derivatized to first order derivative with the zero crossing points of Tolperisone has maxima at 243nm and Paracetamol has maxima at 261nm.The % recovery was found in the range 99.0 ± 0.012, 100 ± 0.342.The developed method was validated statically by recovery studies. The %R.S.D was found to be less than 2. Thus the proposed method was simple, precise, economic, rapid, accurate and successfully applied for simultaneous determination.

Keywords: Tolperisone (TPE), Paracetamol (PCL), Simultaneous equation, Derivative spectroscopy method.

INTRODUCTION

Tolperisone (TPE) a Piper dine derivative was a centrally-acting muscle relaxant. Typically, TPE is indicated in the treatment of acute muscle spasms in back pain and spasticity in neurological diseases. Its IUPAC name was 2-methyl-1-(4-methylphenyl)-3-(1-piperidyl) propan-1-one with the Molecular formula $C_{16}H_{23}NO$. Paracetamol (PCL) is chemically N (hydroxyl phenyl acetamide) with the Molecular formula $C_8H_9NO_2$. It is used mainly used as antipyretic, a non- opioid and non-salicylate analgesic [4, 5] It is indicated for the treatment of moderate to severe pain. Paracetamol was official in Indian Pharmacopoeia [3] and British Pharmacopoeia. Both the drugs are available in combined tablet dosage form.

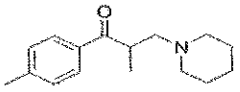


Fig. 1: Chemical structure of Tolperisone

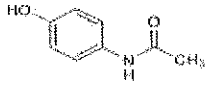


Fig. 2: Chemical structure of Paracetamol

Literature survey reveals that there are UV[6], HPLC[7-11] Capillary electrophoresis[12] methods were reported for the estimation of PCL and for TPE UV[19,20] HPLC methods [17,18] in pharmaceutical formulations in some other combinations and also in single dosage form. The extensive review of the literature revealed that no method was yet reported for the simultaneous estimation of both the drugs in combined dosage forms. This paper describes simple, rapid, accurate, reproducible and economical method for the Simultaneous estimation of TPE and PCL in tablet formulations using derivative method.

MATERIALS AND METHOD

Instrumentation

The present work was carried out on Shimadzu-1700 double beam UV visible spectrophotometer with a pair of 10mm matched quartz cell. Glass wares used were of A grade and soaked overnight in a mixture of chromic acid and sulfuric acid rinsed thoroughly with distilled water and dried in hot air oven. Shimadzu AUX- 200 digital balance.

Reagents and Chemicals

All the chemicals used were of analytical grade and procured from Qualigens, India Ltd. Distilled water of Analytical grade.TPE and PCL were procured as a gift sample from Amaranth pharmaceuticals, Pondicherry. Formulation purchased from Local pharmacy market.

MATERIALS AND METHODS

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 distilled water, used for the analysis of both TPE and PCL for the proposed method.

Preparation of standard stock solution

10 mg of TPE and PCL raw material were weighed and transferred into 10 ml volumetric flasks separately and dissolved in distilled water and made up to the volume with water. These solutions were observed to contain 1000 µg mL^{-1}.And further dilution was made to get concentration of 10 µg mL^{-1}

Selection of wavelength for Estimation

From the stock solutions of TPE and PCL, 10µg mL^{-1} concentration solutions were prepared .The stock solutions were scanned between the wavelength ranges from 200 - 400 nm by using distilled water as blank and the spectra were recorded. From the overlain spectra of TPE and PCL 261nm and 243 nm were selected for the estimation of by Simultaneous equation method. (Fig-3) For Derivative spectroscopy method, the zero order spectra was derivatized to first order derivative spectrum in that 261 nm was selected for the estimation of PCL, (zero crossing for TPE) and 243 nm was selected for the estimation of TPE (zero crossing for PCL) (Fig-4). The Stability was performed by measuring the absorbance of same solution at different time intervals. It was observed that PCL was stable for 3 hours and TPE was stable for more than 3 days at all the selected wavelengths.

Spectral and Linearity Characteristics

The aliquots of stock solution of TPE (0.5-2.5ml of10 µg/ml) and PCL (0.5-2.5ml Of 60µg/ml) were transferred into 10 ml volumetric flasks to get the concentration of (0.5-2.5µg/ml, 3- 9µg/ml) were made up to the volume with distilled water. The absorbance of different concentration solutions were measured at 261, 243, nm in the normal spectrum for simultaneous equation method. The zero order spectra was derivatized to first order derivative spectra with the wavelengths 243 nm, 261 nm (1-5µg/ml, 6-30µg/ml)(zero crossing points for PCL and TPE respectively. The calibration curve was plotted at their corresponding wavelengths. All two drugs TPE

and PCL were found linear with the concentration range of 0.5-2.5 µg/ml and 3-15 µg/ml respectively at their respective wavelengths.

Analysis of marketed formulation

Twenty tablets of formulation (TPE 50 mg and 300 mg of PCL) were weighed accurately. The average weight of tablets were found and powdered. The tablet powder equivalent to 15 mg of TPE was weighed and transferred into 100 ml volumetric flask added a minimum quantity of distilled water to dissolved the substance by using ultra sonication for 15 minutes and made up to the volume with the same (1000 µg mL^{-1}). The content was filtered through whatman filter paper No. 41. From the cleared solution, further dilutions were made by diluting 1 ml to 10ml volumetric flask, further diluted 1 ml to 10 ml to obtain 1.5 µg mL^{-1} of TPE which contains 9µg mL^{-1} of PCL theoretically. The absorbance measurements were made 6 times for the formulation at 261 nm, 243 nm, in normal spectrum and 243nm and 261 nm. For the first order derivative spectrum, 2ml 0f 1.5µg mL^{-1} into 10ml standard flask contains 3µg mL^{-1} of TPE and18µg mL^{-1} of PCL theorectically.From the absorptivity values of TPE and PCL at 261 nm, 243 nm, the amount of TPE and PCL were determined by using Simultaneous equation method and Derivative spectroscopic method.

Method A: Simultaneous Equation Method

From the standard preparation, various dilutions were made at concentration range from 0.5-2.5µg/ml and 3-15µg/ml. It was observed that it obeys the Beer's law.

The simultaneous equations formed were,

At λ_1 $A_1 = ax_1bc_x + ay_1bc_y$ ------ (1)

$A1 = 628\ C_X + 376\ C_Y$ ------ (2)

At λ_2 $A_2 = ax_2bc_x + ay_2bc_y$ ------- (3)

$A2 = 288C_X + 730C_Y$------ (4)

Were A1 and A_2 are the absorbance of sample solution at 261 and 243 nm respectively. Cx and C_Y are the concentration of TPE and PCL respectively (µg /ml) in sample solution.

The absorbance's (A_1& A_2) of the sample solution were recorded at 261 and 243nm respectively and concentration of both the drugs were calculated using above mentioned equation (2&4). Precision of the method was determined by carrying out Intra-Day (n = 3) and Inter- Day (n = 3) studies.

Method B: Derivative Spectroscopy Determination

UV spectra of both the drugs (TPE and PCL) were derivatized to first order derivative with $\Delta\lambda$ = 1 for the entire spectrum. Zero crossing points for TPE and PCL were found to be 261nm and 243 nm respectively (Fig 4). From the above stock solution, aliquots were drawn and suitably diluted so as to get the final concentration range of 1-6 µg/ml of TPE and 6-30 µg/ml of PCL and the readings were taken in the first order mode at the selected wavelengths. Optical and regression data were calculated. Accuracy of the method was checked by preparing five mixed standards containing different concentration, absorbance's were measured at respective zero crossing points in first order UV spectrum and amount present in the sample was calculated from their respective calibration curve. Precision of the method was determined by performing Intra -Day (n = 3) and Inter- Day (n = 3).

Recovery studies

The recovery experiment was done by adding known concentrations of TPE and PCL raw material to the 50% pre-analyzed formulation. Standard TPE and PCL in the range of 80 %, 100 % and 120% are added to the 50% pre-analyzed formulation into a series of 10 ml volumetric flasks , dissolved with distilled water and made up to the mark with the same. The contents were sonicated for 15minutes. 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 TPE and PCL respectively. The amount of each drug recovered from the formulation was calculated for all the drugs by Simultaneous Equation method, and Derivative spectroscopic method. The procedure was repeated for three times

Validation of developed method

Linearity

A calibration curve was plotted as concentration vs. absorbance. TPE was found to be linear in the concentration range of 0.5 to 2.5 µg/ ml at 261 nm, PCL was found to be linear in the concentration range of 3 to 15 µg/ ml at 243 nm.

Precision

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

Ruggedness

Ruggedness of the method was confirmed by the analysis of formulation performed in different instrument and also by the different analysts. The amount and % RSD were calculated.

Accuracy

Accuracy of the method was confirmed by recovery studies. To the preanalyzed formulation, known quantities of raw materials of TPE and PCL 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 found to be 0.994798

LOD and LOQ

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

RESULTS AND DISCUSSION

A simple accurate and precise simultaneous equation method was developed and validated. The drug samples were identified by melting point check and IR spectrum. The solubility of PCL and TPE were determined as per I.P specifications. Trials were made with a variety of polar and non-polar solvents. From the solubility profile double distilled water were the common solvents for both the drugs.

10 µg/ ml concentrations of these two drugs were scanned in the UV region and the spectra were recorded. From the spectra the λ max of the drugs were found to be 261 nm for TPE, 243 nm for PCL. The zero order spectra were derivatized to first order derivative spectra with zero crossing at 261nm for TPE and 243nm zero crossing for PCL respectively. The spectra for TPE and PCL are shown in figure 3 and 4 respectively. .

The stability of the drugs was studied by measuring the absorbances at different time intervals. All the drugs are stable more than 3 hrs in distilled water.. Various aliquots of TPE & PCL in water were prepared for both simultaneous and derivative method in the concentration range of 0.5 – 2.5 µg/ ml, 3 – 15 µg/ ml respectively and the absorbance of those solutions were measured. The calibration curve was constructed. The preparation of calibration curve was repeated in six times for each drug at their selected wavelengths. The optical parameters like, sand ell's sensitivity, molar absorptivity, correlation coefficient, slope, intercept, LOD and LOQ were calculated. The correlation coefficient for both 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 calibration curve was plotted using concentration against absorbance. To confirm the precision of the method, the analysis of formulation was repeated in six times. The

amount present in tablet formulation was in good concord with the label claim and the % RSD values were found to be 0 .994798, 0.130619 and 0.6679 for TPE and PCL respectively. The low % RSD values indicate that the method has good precision. The results of analysis are shown in table 3.

The intermediate 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 on three consecutive days. The % RSD value of intraday and inter-day analysis was found to be 0.37990 and 0.23915 for TPE, 0.915402 and 0.647017 for PCL. The results showed that the less % RSD value and it were confirmed that the intermediate precision of the method was good.

The developed method was also validated for ruggedness. It refers to the specificity of one lab to multiple days which may include different analysts, different instruments and different sources of reagents and so on. In the present work, it was confirmed by different analysts and by different instruments. The low % RSD values indicate that the developed method was more rugged. The results were shown in table

The accuracy of the method was confirmed by recovery studies. To the pre analyzed formulation, a known quantity of TPE and PCL raw material were added at different concentration 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 98.98 – 100.77% for TPE, 99.28 to 100.45% for PCL. The low % RSD value for three drugs indicates that this method is very accurate. The recovery data's were shown in table 3. The high percentage recovery revealed that no interference produced due to the excipients used in formulation. Therefore, the developed method was found to be accurate.

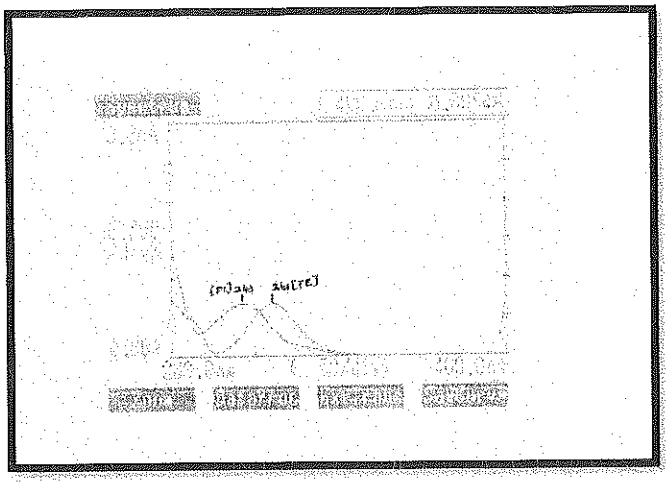


Fig. 3: Overlain Spectrum of TPE and PCL

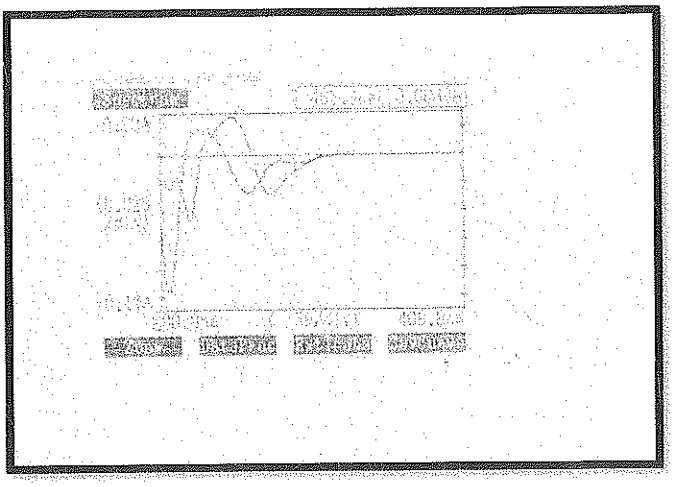


Fig. 4: Overlain Spectrum of Derivative Spectroscopy

Table 1: Optical Characteristics

Parameters	Method A		Method B			
	TPE	PCL	TPE	PCL	TPE	PCL
λ max (nm)	261nm	243nm	261nm	243nm	243nm	261nm
Beer's law limi	0.5-2.5	0.5-2.5	3-15	3-15	1-6	6-30
Sandell's sensitivity)	0.01587877	0.035098275	0.02717321	0.0137006	0.45632338	0.39407018
Molar absorptivity	17698.96	8116.41	5683.616	11034.68	129.6372	384.05283
Correlation coefficient (r)	0.99984	0.999638	0.9998	0.9995	0.99912	0.99965
Slope(m)	0.62977	0.0284914	0.036800952	0.072992	0.00219	0.00253
Intercept (c)	0.00010476	0.0001523	0.001942	0.00059047	0.0001047	1.9047
Standard error	0.00036436	0.00253358	0.0042700	0.001411	0.45632	0.0001385

Table 2: Quantification of tablet formulation

Drug		Label Claim	Amount Found	S.D	R.S.D	S.E
MET A	TPE	50mg	49.9mg	0.983192	0.994798	0.027311
	PCL	300mg	299.9mg	0.13084	0.130619	0.005215
MET B	TPE	50mg	49.86mg	0.07071	0.070534	0.17675
	PCL	300mg	298.9mg	0296985	0.299259	0.017873

Table 3: Recovery Studies

Methods		%	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.
MET A	TPE	80	1.5	1.2	2.7	2.699	99.60	0.36501	0.37018	0.04056
		100	1.5	1.5	3.0	4.5112	98.58	0.60929	0.61802	0.00677
		120	1.5	1.8	3.3	5.019	99.06	0.37207	0.37558	0.04134
	PCL	80	9	7.2	16.2	15.990	100.92	1.15725	1.14663	0.12858
		100	9	9	18	18.0109	101.57	1.90616	1.86927	0.2118
		120	9	10.8	19.8	19.601	99.49	1.28204	1.28004	0.1424
MET B	TPE	80	3	2.4	5.4	5.3961	99.8	0.378153	0.03799	0.01215
		100	3	3	6	6.001	100.01	0.238747	0.23915	0.00955
		120	3	3.6	9.6	9.5962	99.06	0.909776	0.91543	0.0.252
	PCL	80	18	14.4	32.4	32.145	100.92	0.0112	0.1005	0..0241
		100	18	18	36	35.9969	99.86	0.003714	0.3689	0.3265
		120	18	21.6	39.6	38.8962	98.42	0.00123	0.1526	0.1.002

Table 4: Intermediate precision and rugedness of method

Parameters	Label claim Estimated (method-a) TPE PCL	Label claim Estimated (method-b) TPE PCL
Intra day	100.01 100.05	99.5 100.01
Interday	101.02 99.26	98.23 99.52
Instrument -1	99.82 97.41	98.74 100.30
Instrument -1	98.76 101.25	99.99 98.52
Analyst-1	98.65 100.36	100.21 99.65
Analyst-1	100.54 99.85	100.10 98.71

CONCLUSION

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 TPE and PCL.

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.

ACKNOWLEDGEMENT

The authors wish to thank Sakthi Arul ThiruAmma and Thirumathi Amma ACMEC Trust, for providing facilities to do the work in successful manner. We are grateful to Amaranth pharmaceuticals, Pondicherry for providing gift sample to carry the work.

REERENCES

1. Text on validation of Analytical procedures, ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, 27, **1994**, 1-5.
2. Validation of Analytical procedures; Methodology, ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, **1996**, 1-8.
3. The Indian Pharmacopoeia. The Controller of Publication, Ghaziabad, Vol.II, **2007**, 701.
4. The Merck Index. 14th edn, Merck Research Laboratories Division of Merck & Co. Inc, USA, **2006**, 4389(756), 386(66).
5. The United States Pharmacopoeia, 22nd Revision United States Pharmacopoeia Convention, Inc., Rock villa, MC. USA, **1995**, 1776-1777.
6. Beckett, A.H. and Stenlake, J.B. Practical pharmaceutical chemistry. 4th Edition- part II, CBS Publishers and Distributors, New Delhi, **2007**, 278-300, 307-312.
7. Chatwal, R. Gurdeep. And Sham K. Anand. Instrumental Methods of Chemical Analysis, 5th revised edition, Himalaya Publication House, Mumbai, **2008**, 2.107 – 2.120.
8. Devala rao, G. A textbook of pharmaceutical analysis, 1st edn., vol II, Birla publications pvt Ltd, **2004**, 1.
9. Wadher SJ, Pathankar PR, Manisha Puranik, Ganjiwale RO, Yeole PG: Simultaneous spectrophotometric estimation of Paracetamol and Metoclopromide HCl in solid dosage form. Indian J of PharmSci **2008,** 70 (3): 393-395.
10. Ghada M, Hadad, Samy Emara, Waleed, Mahmand MM: Stability indicating RP-HPLC method for determination of Paracetamol with dantrolene and Cetrizine and Pseudoephedrine in two pharmaceutical dosage forms. Talanta **2009**, 79: 1360-1367.
11. Udupa N, Karthik A, Subramanian G, Ranjith Kumar A: Simultaneous estimation of Paracetamol and Domperidone by HPLC method. Indian J Pharm Sci **2007**, 69 (1), 140-144.
12. Subramanian G, Vasudevan M, Ravishankar S, Suresh B: Validation of RP-HPLC method for simultaneous determination of Paracetamol, Methocarbamol, Diclofenac potassium in tablets. Indian J Pharm Sci **2005,** 67 (2): 260-263.
13. Fijalek Z, Wyszecka-Kaszuba E, Warowna-Grzeskiewicz M: HPLC with amperometric detection for the determination of 4-aminophenol, the main impurity of Paracetamol in multicomponent analgesic preparation. J Pharm Biomed Anal **2003,** 32: 1081-1086.
14. Lotfi Monser, Frida Darghouth: Simultaneous LC determination of Paracetamol and related compound in pharmaceutical formulation using carbon based column. J of Pharm Biomed Anal. **2002**, 27.
15. Shulin Zahao, Dan Xiao, Wenling Bai, Hongyan Yuan: Capillary electrophoresis with chemiluminescence detection of Paracetamol. Anal Chim Acta **2006**, 559: 195-199.
16. Hofer D, Lohberger B, Steinecker B, Schmidt K, Quasthoff S, Schreibmayer W

17. **(2006)**"*EurJPharmacol* **538** (13):.doi:10.1016/j.ejphar.2006.03.034. PMID 16650844
18. Jung-Woo Bae, Young-Seo Park, Uy-Dong Sohn, Chang-Sun Myung, Byung-Kwon Ryu, Choon- Gon Jang, and Seok-Yong Lee, Arch Pharm Res Vol 29, No 4, 339-342
19. Napaporn Youngvises, Boonsom Liawruangrath and Saisunee Liawruangrath, Journal of Chromatography.
20. R.Prashanthi, V.Jagathi, M.Shaiba, K.Raghavi, M.Sindhura, IJPI's Journal of Analytical Chemistry ISSN 2229 – 6867,**2011**
21. Mit J Patel, R Badmanaban, CN Patel Pharmaceutical methods : Vol : 2 | Issue : 2 | 124-129 **2011**
22. R.Prashanthi, V.Jagathi, M.Shaiba, K.Raghavi, M.Sindhura Asian journal of chemistry Volume 04, Issue 02, 2011

G.Abirami et al. / Journal of Pharmacy Research 2012,5(12),5549-5552

Research Article
ISSN: 0974-6943

Available online through
www.jpronline.info

Development and validation of spectrophotometric method for estimation of Sitagliptin phosphate and Simvastatin in combined dosage form by derivative spectrophotometry

G.Abirami*, Dr.T.Vetrichelvan

Department of pharmaceutical analysis,Adhiparasakthi College of Pharmacy,Melmaruvathur-603306, Andhra Pradesh, India

Received on:14-07-2012; Revised on: 19-08-2012; Accepted on:17-09-2012

ABSTRACT

Novel combination of Sitagliptin phosphate (SITA) and Simvastatin (SIMV) is available as combined tablet dosage form in the ratio 10:4 and no spectrophotometric method has been reported yet. Recently, a combination of SIM and SITA has been launched in the market. The present research work aims to develop a simple, sensitive, accurate reproducible method for the simultaneous estimation of both the drugs by first order derivative spectrophotometric method, using methanol as a solvent. The method was performed at 238 nm (zero crossing point of sitagliptin) and 277 nm (zero crossing point of simvastatin) respectively. The regression analysis data for the calibration plot showed good linear relationship in the concentration range of 10-50µg/ml for sitagliptin phosphate and 4-20 µg/ml respectively for simvastatin. The average percentage recovery of sitagliptin phosphate and simvastatin combination was found to be100 ±0.304 respectively. The LODs for sitagliptin phosphate and simvastatin were 1.638105 and 0.27292 and LOQs were found to be 0.49635679 and 0.8270138 respectively. Statistical analysis proves that the method is reproducible and selective for simultaneous determination of sitagliptin phosphate and simvastatin .The results were found to be within acceptance criteria according to ICH Guidelines.

Key words:Sitagliptin Phosphate (SITA) and Simvastatin (SIMV), Simultaneous Estimation First order Derivative Spectrophotometric method.

INTRODUCTION

Simvastatin(SIMV) is chemically 2,2-Dimethylbutanoic acid (1S,3R,7S, 8S,8aR) 1,2,3,7,8,8ahexahydro-3,7-dimethyl-8-[2-[(2R,4R)-tetrahydro-4-hydroxy-6 oxo2H pyran-2yl]ethyl]1-napthalenyl ester used as a HMG-CoA reductase inhibitors SIM is official in Indian Pharmacopoeia and SITA is official in USP. Sitagliptinphosphate7-[(3R)-3-amino-1-oxo-4-(2,4,5trifluorophenyl)butyl]-5,6,7,8-tetrahydro-3-(trifluoromethyl)-1,2,4-triazolo[4,3-a]pyrazine phosphate used as an oral anti hyperglycemic of the di peptidyl peptidase-4 (DPP-4) inhibitor class. This enzyme-inhibiting drug is used either alone or in combination with other oral anti hyperglycemic agents (such as metformin or thiazolidinedione) for treatment of diabetes mellitus type 2. The benefit of this drug is its lower side-effects (e.g., less hypoglycemia, less weight gain) in the control of blood glucose values. By the literature survey UP[9,20,21] HPLC[7,8] Stability Indicating HPLC, LC-MS[13,14] methods have been reported for the estimation of SIM while LC-MS methods have been reported and no other analytical methods have been reported for SITA. Moreover the literature survey revealed that so far, no method has been reported for estimation of SIMV and SITA in combined dosage form by simultaneous equation methods using UV spectroscopy. Therefore the present research work aims to develop a simple, sensitive, accurate and reproducible method for simultaneous estimation of sitagliptin phosphate and simvastatin in combined dosage form by first order derivative spectrophotometric method.

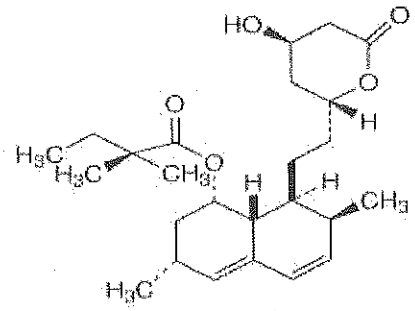


Fig-1 Chemical structure of Simvastatin

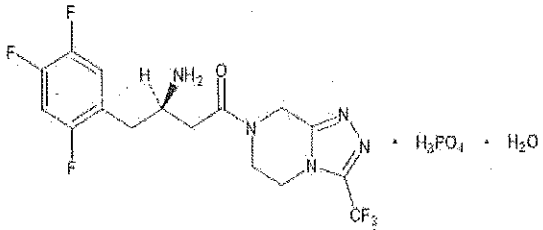


Fig-2 Chemical structure of Sitagliptin Phosphate

***Corresponding author.**
G.Abirami M.Pharm.,
Assistant professor
Department of pharmaceutical analysis
Adhiparasakthi College of Pharmacy
Melmaruvathur-603306,
Andhra Pradesh, India

MATERIALS AND METHODS

Instruments

Only AR grade reagents and solvents were used. The pure drug of sitagliptin phosphate and simvastatin were obtained as a gift sample from Alkem laboratories, Hyderabad. The spectrophotometer Shimadzu 1800 model was used. All the apparatus and instruments were calibrated and validated as per cali-

bration and validation protocol specified before starting the experimental work.

Standard Stock solution

The standard stock solutions of SITA and SIMV were prepared by dissolving 10mg each of sitagliptin phosphate and simvastatin in methanol as solvent and final volume was adjusted to get a concentration of 10 µg/ml of each and the solutions were scanned in the UV region of 200-400nm respectively. The zero order spectra were obtained and it was derivatised to first order spectrum. The values of amplitudes were measured for sitagliptin phosphate at 277nm (zero crossing point at 238nm) and 238nm measured for Simvastatin (zero crossing point at 277nm) respectively. A calibration curve was constructed and regression equation was obtained for each drug. The Stability was performed by measuring the absorbance of same solution at different time intervals. It was observed that SITA and SIMV were stable for 24 hours.

Spectral and Linearity Characteristics

The aliquots of stock solution of SITA (0.5-2.5ml of10 µg/ml) and SIM (1-5ml 0f 60µg/ml) were transferred into 10 ml volumetric flasks to get the concentration of 10-50µg/ml, 4- 20µg/ml were made up to the volume with methanol. The zero order spectra were derivatized to first order derivative spectra with the wavelengths 238 nm, 277 nm (zero crossing points for SITA and SIMV) respectively. A calibration curve was constructed and regression equation was obtained for each drug.

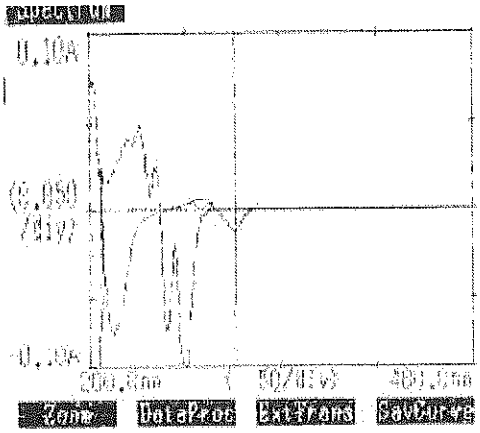


Fig-1 Overlaid First Order Derivative Spectra

Preparation of Sample Solution

Twenty tablets (Juvisync) were weighed accurately and a quantity of tablet powder equivalent to10 mg was transferred into 100 ml volumetric flask added a minimum quantity of methanol to dissolved the substance by using ultra sonication for 15 minutes, and made up the volume to 100ml volumetric flask. Then the content was filtered through what man filter paper No. 41. From the cleared solution, further dilutions were made by diluting 3 ml to 10ml volumetric flask to obtain 30 µg mL^{-1} of SITA which contains 12µg mL^{-1} of SIMV theoretically. The absorbance measurements were made 6 times for the formulation by derivatising the zero order spectra into first order derivative spectrum at 238 nm, 277 nm respectively.

Validation Parameter of The Developed Methods

Validation of the developed method was carried out as per ICH Guidelines. Parameters such as Linearity, Accuracy, Precision, LODs and LOQs were taken up as tests for method validation.

Linearity

For Quantitative analysis of sitagliptin phosphate and simvastatin, linearity curve was plotted. Linearity range of sitagliptin phosphate and simvastatin was established in the concentration range of (10-50µg/ml, 4-20µg/ml) respectively. The slope and intercept along with its correlation coefficient is given in the Table-1.

Precision

The Inter day and intraday variations for determination of SITA and SIM were carried out five times in the same day and five consecutive days and % RSD were calculated. The method was found to be precise due to low values of the %RSD. The precision of an analytical method is determined by assaying a sufficient number of aliquots of a homogeneous sample to be able to calculate statistically valid estimate of % Relative Standard Deviation (%RSD). Intermediate precision was done to express within laboratory variation, on different days. The working standard mixture and sample solution were analyzed %RSD was found to be less than 2%.

LODs and LOQs

The LODs and LOQs of developed method were studied as per ICH Guidelines. Several approaches for determining the LODs and LOQs are possible, depending on the procedure i.e. a non-instrumental or instrumental.

$$LODs = 3.3\ \sigma/S$$

$$LOQs = 10\ \sigma/S$$

Where σ = standard deviation of response, s = slope of calibration curve

The results obtained are shown in the Table-1

Table -1 Optical Characteristic

Parameters	SITA	SIMV
Detection wavelength (nm)	277nm	238nm
Beer's law limit (µg mL^{-1})	10-50µg/ml	4- 20µg/ml
Correlation coefficient (r)	0.999301635	0.9991315344
Régression équation (y=mx+c)	0.000401714x+0.000357143	0.001511429x+0.000252381
Slope (m)	000401714	001511429
Intercept (c)	0.000357143	0.000252381
LOD (µg mL^{-1})	1.63810574	0.272922
LOQ (µg mL^{-1})	49635679	0.8270138
Standard Error	0.000314188	0.000468178

Robustness

The Robustness was started by analyzing the same samples of SIM and SITA by deliberate variation in the method parameters. The' change in responses of SITA and SIM were noted. Robustness of the method was studied by apparatus and changing solvent ratio.

Specificity

Results of tablet solution showed that there is no interference of the excipients when compared with the working standard solution. Thus, the method was said to be specific.

Table -2 Assay of formulation

S.No	Label claim		Amount found		% Label claim	
	SITA	SIMV	SITA	SIMV	SITA	SIMV
1	100	40	99.8210	40.1909	99.82	100.10
2	100	40	98.6242	40.6402	98.62	101.60
3	100	40	100.321	40.2124	100.3	100.5
4	100	40	99.9368	40.3026	99.9	100.7
5	100	40	99.9750	40.0271	99.9	100.05
6	100	40	99.8900	39.911	99.8	100.02

Recovery studies

Accuracy of the method was determined in terms of % recovery of standard. Recovery studies were carried out by addition of standard drug solution at the level of 80% 100% and 120% to the 50% pre analyzed sample. Results of the recovery study were found to be within the acceptance criteria 100± 10%, indicating a good degree of sensitivity of the method towards detection

of analytes in sample. In this method the known concentration standard drug was added to the assay sample. The amount present was calculated and the assay amount was reduced from it, which gives the amount recovered. The average %recoveries for SITA and SIM were obtained shown in the Table -3

Table-3 Recovery Studies

Drug	Recovery level	% Recovery	%R.S.D	S.D	S.E
SITA	80%	99.9262			
	100%	100.340	0.199202	0.199422	0.22158
	120%	99.95			
	80%	100.125			
SIMV	100%	100.25	0.124844	0.124844	0.13889
	120%	100.902			

RESULT AND DISCUSSION

The UV spectrum of SIM exhibited three well defined peaks at 233nm, 238nm and 247nm and virtually no absorbance above 259nm where SITA exhibited a broad peak covering 268nm as broad peak. Hence multicomponent analytical method may not be possible for the simultaneous estimation method. Hence alternative method for SIM and SITA is by using derivative spectroscopic method Under experimental conditions described, calibration curve, assay of tablets, recovery studies, precision studies, LODs &LOQs were performed. Using appropriate dilutions of standard stock solution, the two solutions were scanned separately. A critical evaluation of proposed method was performed statistical analysis of data where slope intercept correlation coefficient was studied. Beer's law obeys in the concentration range of 10-50µg/ml, 4- 20µg/ml for each drug and correlation coefficient of 0.99937 and 0.99972 for SIM &SITA. The proposed method was also evaluated by assay of commercially available tablets containing SIM &SITA (n=6). The % recovery assay was found to be 100.8 & for SIM and 101.6% for SITA, the recovery study results ranged from 100 ± 0.125 and %RSD value is 0.199202. The accuracy and reproducibility is evident from the data as results are close to100.5% and standard deviation is low.

Table-4 Ruggedness Study

Drug	Condition	Average* % Obtained	S.D	%R.S.D	S.E.
SITA	Analyst 1	100.55	0.357771	0.355814	0.009938
	Analyst 2	100.68	0.408607	0.405848	0.01135
	Instrument 1	100.35	0.742994	0.740400	0.020639
	Instrument2	100.42	0.823452	0.814325	0.256732
SIMV	Analyst 1	101.43	0650723	0.641549	0.018076
	Analyst 2	100.15	1.04022	1.038575	0.028895
	Instrument 1	100.37	1.484687	1.479092	0.041241
	Instrument2	100.31	1.342576	1.43215	0.397654

CONCLUSION

The above method does not suffer from any interference due to common excipients. It indicates that method was accurate. Therefore the proposed method could be successfully applied to estimate commercial pharmaceutical products containing SITA and SIM. 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.

ACKNOWLEDGEMENT

The authors wish to thank **Sakthi Arul Thiru Amma and Thirumathi Amma ACMEC Trust,** for providing facilities to do the work in successful manner. We are grateful to Alkem Laboratories, Hyderabad for providing gift sample to carry the work.

REFERENCES

1. Beckett A.H. and Stenlake J.B. Practical pharmaceutical chemistry. 4th Edition- part II, CBS Publishers and Distributors, New Delhi, **2007**, 278-300, 307-312.
2. Chatwal R. Gurdeep and Sham K. Anand. Instrumental Methods of Chemical Analysis, 5th revised edition, Himalaya Publication House, Mumbai, **2008**, 2.107 – 2.120.
3. Jag Mohan, Organic Spectroscopy (principles and Application), 2nd edition, 127
4. Kellner R, Mermet J.M, Otto M, Valcarcel M. and Widmer H.M. A modern approach to analytical science, 2nd edition, **2004**, 3.
5. Khare R.P. Analysis instrumentation an introduction, CBS Publishers & Distributors. **2007**, 1
6. Morris M J. et al. Determination of the HMG-CoA Reductase Inhibitors Simvastatin, Lovastatin, and Pravastatin in Plasma by Gas Chromatography/Chemical Ionization Mass Spectrometry. Biol Mass Spectrum, 22, **1993**, 1-8.
7. Tan L, Yang LL, Zhang X, Yuan YS and Ling SS.Determination of Simvastatin in Human Plasma High Performance Liquid Chromatography. Se Pu. 18, **2000**, 232-234.
8. Curlucci G, Mazzeo P, Biordi L, and Bologna M. Simultaneous Determination of Simvastatin and Hydroxy Acid Form in Human Plasma by High Performance Liquid Chromatography with UP Detection. J Pharm Biomed Anal, 10, **1992**, 693-7
9. Wang L and Asgharnejad M. Second- Derivative Spectrometric Determination of Simvastatin in Tablet Dosage Form. J Pharm Biomed Anal, 21, **2000**, 1243-124
10. Tan L, Yang LL, Zhang X, YuanYS, and Ling SS. Determination of simvastatin in human plasma by high performance liquid chromatography. Se Pu. **2000**; 18(3):232-2
11. Curlucci G, Mazzeo P, Biordi L,Bologna M. Simultaneous determination of simvastatin and its hydroxy aciform in human plasma by high performance liquid chromatography with UV detection. J Pharm BiomedAnal. **1992**; 10(9):693-7
12. Nilesh Jain. et al Spectrophotometric method for simultaneous estimation of simvastatin and ezetimibe in bulk drug and its combine Dosage form Anal21(6):121248S
13. Ochiai H. et al Determination of Simvastatin and Its Active Metabolites in Human Plasma by Column-Switching High Performance Liquid Chromatography of. J Chromatogr B Biomed Sci. **1997**: 694:211-217.).
14. Oswald S, Scheuch E, Cascorbid I, Siegmund W. Ion pairing RP_HPLC analytical method for simultaneous estimation of simvastatin and its α-hydroxy acid. J Chromatogr B, **2006, 830,** 143-150.
15. Zeng Wei et al. have reported Sitagliptin in human urine and hemodialysate using turbulent flow online extraction and tandem mass spectroscopy.
16. PK Sahoo et al. have reported Simultaneous estimation of metformin hydrochloride and pioglitazone hydrochloride by HPTLC method from combined tablet dosage form.
17. Kyoung Soo Lim et al. have reported Pharmacokinetics, pharmacodynamics, and tolerability of the dipeptidyl peptidase IV inhibitor LC15-0444 in healthy Korean men: A dose—block-randomized, double-blind, placebo-controlled, ascending single-dose, phase I study.
18. Ramakrishna Nirogi et al. have reported Sensitive liquid chromatography tandem mass spectrometry method for the quantification of sitagliptin, a DPP-4 inhibitor, in human plasma using liquid-liquid extraction.
19. Tesfaye Biftu et al. Have reported rational design of a novel, potent, and orally bioavailable cyclohexylamine DPP-4 inhibitor by

application of molecular modeling and X-ray crystallography of Sitagliptin.
20. Wang L,Asgharnejad M.SecondderivativeUV spectrometric determination of simvastatin intablet dosage form. J Pharm Biome
21. Sistla R,. Simultaneous Determination of Simvastatin and Ezetimibe in Tablets by HPLC .J Pharm Biomed Anal., **2005**, 39, 517.
22. International Conference on Harmonization (1996). Validation of analytical procedures: Test and methodology. ICH, London.
23. Willard, H., Meritt, L.L. Dean. And Settle. Instrumental Methods of Analysis 7th edition, CBS Publishers and Distributors, New Delhi, **1986**, 592-600.
24. Text on validation of Analytical procedures. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, 27, **1994**, 1-5.
25. Anonymous. Code Q2B, Validation of Analytical procedures; Methodology. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, **1996**, 1-8.

Source of support: Nil, Conflict of interest: None Declared

BIBLIOGRAPHY

8. BIBLIOGRAPHY

1. Swarbrick James and Boylan James, Encyclopedia of Pharmaceutical Technology, Volume I, Marcel Dekker Inc, New York, **(1998),** 217 - 224.
2. Beckett,A.H, Stenlake J.B, Practical Pharmaceutical Chemistry, 4th edition, Part 2, CBS Publishers and distributors, **(1997),** 157-163, 275-337.
3. http://hplc.chem.shu.edu/NEW/HPLC_Book.
4. Szepei Gabor, HPLC in pharmaceutical Analysis, Volume I, **(1990)**, 101-173.
5. Jeffery G.H, Bassett J, Vogel's textbook of Quantitative Chemical Analysis, 5th edition, **(1991)**, 217-235.
6. www.pharmaarticles.net/exclusive/technical/basic-principles-of-hptlc.html.
7. Sethi P. D., HPTLC – High Performance Thin Layer Chromatography, First Edition, CBS Publisher, New Delhi, **1996**, 4-66.
8. Validation of Analytical Procedures: Methodology, ICH Harmonised Tripartite Guidelines, **(1996)**, 1-8.
9. Willard Hobart. H., Merritt L.L., Dean John. A., Instrumental Methods of Analysis, 7th edition, CBS Publishers, 580-610.
10. United States Pharmacopoeia and National Formulary, (24th) Asian Edition, The United States Pharmacopoeia Convention Inc., U.S.A., 3557.
11. Quality Assurance of Pharmaceuticals, (A compendium of guidelines and related materials), **(1997)**, Volume I, WHO, Geneva, 119-124.
12. *(www.askaboutvalidation.com/forum/showthread.php?t=1175)*
13. Rashmin. An Introduction to Analytical Method Development for Pharmaceutical Formulations. Pharmaceutical Reviews. **2008**, 6 (4).

14. Mendham J, Denny R.C, Jeffery G.H and Thomas. Vogel's Text book of Quantitative Chemical Analysis, 5th edition, Longman Publishers, UK, **1994,** 10 - 11.

15. Robert D.Braun. Introduction to Instrumental Analysis, Pharma Book Syndicate, Hyderabad, **2006,**136

16. Tripathi K.D. Essentials of Medical Pharmacology, 6th edition, Jaypee Publications, New Delhi, **2008,** 143, 196, 205

17. Douglas A. Skoog, James Holler F and Timothy A. Nieman. Principles of Instrumental Analysis, 5th edition, Thomson Business Information India Pvt. Ltd., New Delhi, **2006,** 331

18. The British Pharmacopoeia Volume I, International edition, Office of British Pharmacopoeia Commission, London, **2009,** 265.

19. The Indian Pharmacopoeia Volume I and Volume II, The Indian Pharmacopoeia Commission, 3rd edition, Ghaziabad, **2007**,359,450.

20. **(**www.drugbank.com)

21. The Merck Index, 14th edition, Merck Research Laboratories a Division of Merck & Co. Inc, White house Station, NJ, USA, **2006**, 1465, and 1591.

22. William Kemp. Organic Spectroscopy, 3rd edition, Palgrave Publishers, New York, **2006,** 257-258.

23. Gupta S.C. and Kapoor V.K. Fundamentals of Mathematical Statistics, 9th Edition, Sultan Chand and Sons, New Delhi, **1995**, 2.6, 3.2 – 3.28.

24. Martindale, The Extra Pharmacopoeia, 30th edition, The Pharmaceutical Press, London, **1993,** 743, 933, 1210.

25. Shimadzu Instruction Manual AX - 200 Digital Balance, Shimadzu Corporation, Kyoto, Japan, **2001,** 42.

26. Elico Instruction Manual Pharamaspec SL – 210 Series Operation Guide, Elico Limited, Hyderabad, India, **2009**, C – 2.

27. Shimadzu Instruction Manual Pharamaspec UV – 1700 Series Operation Guide, Shimadzu Corporation, Kyoto, Japan, **2001**, 6.2.

28. Code Q2A, Text on Validation of Analytical Procedures. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, and 27th October, **1994,** 1 - 5.

29. Code Q2B, Validation of Analytical Procedures; Methodology. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, 6th November, **1996**, 1 – 8.

30. Guredeep R Chatwal and Sham K Anand. Instrumental Methods of Chemical Analysis, 5th revised edition, Himalaya Publishing House, Mumbai, **2008,** 2.107 - 2.148

31. Joel G. Hardman, Lee E. Limbird and Alfred Goodman Gilman. Goodman and Gillman's. The Pharmacological Basis of Therapeutics, 10th edition, McGraw Hill Medical Publishing Division, New Delhi, **2001,** 741, 1939t, 1984t.

32. Sharma B.K. Instrumental Methods of Chemical Analysis, 25th edition, Goel Publishing House, Meerut, **2006,** S 68 – S 192.

33. Fifeld F.W. and Kealey D. Principles and Practice of Analytical Chemistry, 5th edition, Blackwell Publishers, London, **2004,** 5.

34. Hariyani Kaushik P. Patel, Sathhish. A. Spectrophotometric method for Simultaneous estimation of Tolperisone hydrochloride and Diclofenac sodium in synthetic mixture. International Research Journal of Pharmacy, **2012**, 3(9), 162-165.

35. Monali Patel, Dr.Ragin Shah, Hiren Kadikar. Method development and statistical validation UV spectrophotometric method for Estimation of Tolperisone hydrochloride and Paracetamol in synthetic mixture and combined Dosage form. International Journal of Pharmaceutical Research and Bioscience, **2012,** 1(1)**.**

36. M. G. Patel, R. R. Parmar, P. P. Nayak and D. A. Shah**.** The Simultaneous Estimation of Paracetamol and Tolperisone Hydrochloride in Tablet by UV Spectrophotometric Methods. Journal of Pharmaceutical and Bioscientific Research, **2012,** 2(2), 63-67.

37. Koladiya Bhavesh, Vaghela Vipul M. UV Spectrophotometric Method: A Quantitative Estimation of Tolperisone Hydrochloride in Bulk and Pharmaceutical Dosage Form. International Journal of Pharm Tech Research, **2012**, 4(3), 1317-1322.

38. Carolin Nimila, P.Balan, N.Chiranjeevi, V. Uma Maheswari, S.Rajasekar. Method development and statistical validation of UV spectrophotometric method for Tolperisone hydrochloride in bulk and tablet dosage form. Journal of Pharmacy Research, **2011**, 4(5), 1356-1357.

39. MM Sorathiya, KS Murlikrishna, HJ Vekariya. Simultaneous Estimation of Paracetamol and Tolperisone Hydrochloride in Bulk and Combined Dosage Form by Derivative Spectrophotometric Method. **Inventi Rapid: Pharm Analysis & Quality Assurance, 2012, 1/26.**

40. Mandhanya Mayank, Dubey Nitin, Chaturvedi S.C, Jain D.K. Simultaneously Estimation of Paracetamol, Aceclofenac and Rabeprazole in Tablet Dosage Form Using UV Spectroscopy. Asian Journal of Pharmacy & Life Science, **2011,** 1(2), 113-117.

41. Karuna B. Singh, Shekhar B. Waikar, Suhas P. Padmane. A Validated RP-HPLC Method for the Simultaneous Estimation of Paracetamol and Naproxen in Tablet Formulation. International journal of pharmaceutical sciences and research, **2012,** 3(10), 3742 -3745.

42. Satyanarayana. P. V. V, Murali. M. Simple validated isocratic RP – HPLC method for estimation of Tolperisone in bulk and pharmaceutical dosage form. Scholars Research Library Der Pharma Chemica, **2011,** 3(5), 13-19.

43. P.Sai Praveen, B.Anupama, V.Jagathi, G.Devala Rao. Spectrophotometric determination of Tolperisone using 2, 4-dinitrophenylhydrazine reagent**.** Int. J. Res. Pharm. Sci. **2010**, 1(3), 317-320.

44. Buddha Ratna Shrestha, Raja Ram Pradhananga. Spectrophotometric Method for the Determination of Paracetamol. J. Nepal Chem. Soc, **2009,** 24, 39-44.

45. Dhara J.Patel, Vivek P.Patel. Determination of Paracetamol and Lornoxicam in Tablets by Thin Layer Chromatography Combined with Densitometry. International Journal of Chem Tech Research, **2010,** 2(4), 1929-1932.

46. Patcharawee Nunthanavanit. Simultaneous determination of Paracetamol and its main degradation product in generic Paracetamol tablets using reverse-phase HPLC. Journal Health Research, **2010**, 24(3), 103-106.

47. Godse VP. Deodhar.MN, Bhosale.AV, Sonawane. RA, Sakpal .PS, Borkar DD. Reverse Phase HPLC Method for Determination of Aceclofenac and Paracetamol in Tablet Dosage Form. Asian J. Research Chem, **2009,** 2(1), (37-40).

48. S. R. Pattan, S. G. Jamdar, R. K. Godge, N. S. Dighe, A.V. Daithankar. RP-HPLC Method for Simultaneous Estimation of Paracetamol and Etoricoxib

from Bulk and Tablets. Journal of Chemical and Pharmaceutical Research, **2009,** 1(1), 329-335.

49. C. Barbaset, E. Garcia, A. Garcia. Validation of a HPLC quantification of Acetaminophen, Phenylephrine and Chlorpheniramine in Pharmaceutical formulations: capsules and sachets. Journal of Pharmaceutical and Biomedical Analysis, **2002**, 29, 701–714.

50. Toufik S Mulla, Janhavi R Rao, Savita S Yadav, Vishal. V Bharekar and Milindkumar P. Rajput. Development and validation of HPLC method for simultaneous quantitation of Paracetamol and Dexketoprofen Trometamol in bulk drug and formulation**.** Pharmacie Globale, (IJCP) **2001**, 7(9), 1-4.

51. M. Levent altun. HPLC Method for the Analysis of Paracetamol, Caffeine and Dipyrone. Turk J Chem, **2002**, 26, 521 -528.

52. Prasanna Reddy Battu1et E. Garcia. RP-HPLC Method for Simultaneous Estimation of Paracetamol and Ibuprofen in Tablets**.** Asian J. Research Chem, **2000,** 2(1), 70-72.

53. Safaa M Riad, Mamdouh R Rezk, Ghada Y Mahmoud and Abdel-Aziz El Bayoumi Abdel Aleem. Spectrophotometric Determination of Sitagliptin and Metformin in their Pharmaceutical Formulation. Pharmacie globale, International Journal of Comprehensive Pharmacy, **2012,** 3(5).

54. T. Raja, A. Lakshmana Rao. Validated HPTLC Method For Simultaneous Estimation of Metformin Hydrochloride and Sitagliptin Phosphate in Bulk Drug and Formulation. RASAYAN J.Chem, **2012,** 5(3), 407- 413.

55. Jain Pritam, Chaudhari Amar, Desai Bhargav, Patel Shani, Patel Santsaran, Shimpi Hiren. Development and validation of first order derivative UV-Spectrophotometric method for determination of Sitagliptin in bulk and in

Formulation. International Journal of Drug Development & Research, **2011,** 3(4), 194-198.

56. T. Raja and A. Lakshmana Rao. Validated RP-HPLC Method for Simultaneous Estimation of Metformin Hydrochloride and Sitagliptin Phosphate in Bulk Drug and Pharmaceutical Formulation. IJPCBS, **2012,** 2(4), 696-702**.**

57. Hitesh P. Inamdar, Ashok A. Mhaske. RP-HPLC Method for Simultaneous Determination of Metformin Hydrochloride, Rosiglitazone and Sitagliptin – Application to Commercially Available Drug Products. IJPSR, **2012,** 3(9), 3267-3276.

58. Sheetal Sharma, Nimita Manocha, Priya Bhandari, Sohail Harsoliya, Prabhat Jain. Development of UV- spectrophotometry and RP-HPLC Method and its Validation for Simultaneous Estimation of Sitagliptin Phosphate and Simvastatin in Marketed Formulation. International Journal of Pharmaceutical & Biological Archives, **2012,** 3(3), 673-678.

59. Swati Kupkar, Shailaja Jadhav, Vaibhavi Kunjir, Praveen Chaudhari .Simultaneous estimation of Sitagliptin and Metformin hydrochloride in bulk and dosage form by UV spectrophotometry. Journal of Pharmacy Research, **2012,** 5(1), 580-582.

60. Dhiraj Kumar, R.Lavanya, P.Parijata Reddy, Mandeep Kaur, N.Naveen Kumar. Method development and estimation of Sitagliptin phosphate in bulk and Pharmaceutical dosage forms using UV-VIS spectrophotometer. Journal of Pharmacy Research, **2012**, 5(8), 4421- 4424.

61. Anilkumar Voodikala, Arunakumari Vanka, Saikumar V Simhadri, Srinivasa Rao Atla, Santoshkumar Tata. Validated RP-HPLC Method for

the Simultaneous Estimation of Sitagliptin and Simvastatin in Dosage Forms. International Journal of Chemical and Analytical Science, **2012,** 3(11), 1611-1614.

62. Ankur Kothari, Sheetal Sharma. Development and Validation of Spectrophotometric Method for Simultaneous Estimation of Sitagliptin Phosphate and Simvastatin in Tablet Dosage Form**.** Int J Pharm, **2012**, 2(3), 609-612.

63. Narendra Nyola, Govinda Samy Jeyabalan. Method development of simultaneous estimation of Sitagliptin and Metformin hydrochloride in pure and Tablet dosage form by UV - Vis spectroscopy. World Journal of Pharmacy and Pharmaceutical Sciences, **2012**, 1(4), 1392-1401.

64. Amruta B. Loni, Minal R. Ghante, S. D. Sawant. Simultaneous UV Spectrophotometric Method for Estimation of Sitagliptin phosphate and Metformin hydrochloride in Bulk and Tablet Dosage Form. Der Pharma Chemica, **2012,** 4 (3), 854-859.

65. N.Monila, Ravi Pratap Pulla, Harshini Shabad, V.Swathi, J.Rajasekhar, A.Ramesh, B.Koti Reddy, B.Umashankar. New Extractive Method Development of Sitagliptin Phosphate in API and Its Unit Dosage Forms by Spectrophotometry. IOSR Journal of Pharmacy and Biological Sciences, **2012,** 1(6), 37-40.

66. G Jeyabalan, Narendra Nyola. Analytical Method Development and Validation of Sitagliptin Phosphate Monohydrate in Pure and Tablet Dosage Form by UV-Vis Spectroscopy. RRJPA, **2012,** 1(1), 19-23.

67. Gebremriam Enema, D Gowris Sankar. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Sitagliptin and Simvastatin

in Bulk and Tablet Dosage Forms. Inventi Impact: Pharm Analysis & Quality Assurance, **2012**, 4(15).

68. Parag Pathade, Md Imran, Vinod Bairagi, Yogesh Ahire. Development and Validation of Stability Indicating UV Spectrophotometric Method for the Estimation of Sitagliptin Phosphate in Bulk and Tablet Dosage Form. Journal of Pharmacy Research, **2011**, 4(3), 871-873.

69. Shyamala.M, Mohideen.S, Satyanarayana.T, Ch.NarasimhaRaju, SureshKumar.P, Swetha.K. Validated RP-HPLC For Simultaneous Estimation of Sitagliptin Phosphate and Metformin Hydrochloride In Tablet Dosage Form. American Journal of Pharma Tech Research, **2011,** 1(2), 93-101.

70. A.B. Loni. Method development and validation for simultaneous determination of Sitagliptin phosphate and Metformin hydrochloride by RP-HPLC in bulk and tablet dosage form. Analytical & Bio analytical Techniques, **2011,** November 22-24, Hyderabad, International Convention Centre, India.

71. Radhika Bhaskar, Rahul Bhaskar, Vineet Singla**.** Simultaneous estimation of Simvastatin and Metformin hydrochloride in bulk and solid Dosage forms**.** RASAYAN J.Chem, **2010,** 3(3), 507-513.

72. P Bonde, S Sharma, N Kourav, AM Attar. Development and Validated UV Spectrophotometric and RP-HPLC Methods for the Estimation of Simvastatin and Ezetimibe in Combined Pharmaceutical Dosage Form**.** Inter J Curr Trends Sci Tech, **2010,** 1(3), 135–142.

73. B.Stephen Rathinaraj, V.Rajamanickam, Ch.Rajveer, D.Kumaraswamy, Ganesh Shehraobanglae, A.Arunachalam. Development and Validation of a

HPTLC Method for the Estimation of Simvastatin and Ezetimibe. International Journal of Pharmaceutical & Biological Archives, **2010,** 1(4), 325 – 330.

74. Nagaraju P. Vishnu vardhan Z. A validated reverse phase HPLC method for the simultaneous estimation of Simvastatin and Ezetimibe in pharmaceutical dosage forms. Journal of Global Pharma Technology. **2010,** 2(4), 113-117.

75. Joshi H. V, J. K. Patel, Lata Kothapalli. Simultaneous derivative and multicomponent spectrophotometric determination of Simvastatin and Ezetimibe in tablets. Der Pharma Chemica, **2010,** 2(2), 152-156

76. A. Sunitha, S. Balaji. Development and validation of spectrophotometeric method for simultaneous determination of Simvastatin and Ezetimibe in tablet formulations. Pak. J. Pharm. Sci., **2010,** 23(4), 375-378.

77. Mujeeb Ur Rahman, Gazala Parveen, N.K.Nyola. Simultaneous estimation of Simvastatin and Ezetimibe in pharmaceutical Tablet dosage forms by RP-HPLC: A Review. IJPRD, **2010**, 2(9), 56-62.

78. Jayapal Reddy Samaa, C, Rama Rao Kalakuntlab, V. Surya Narayanac Rao, and P. Reddannaa Simultaneous estimation of Simvastatin and Ezetimibe in pharmaceutical formulations by RP-HPLC method. J. Pharm. Sci. & Res, **2010,** 2 (2), 82-89.

79. Nilesh Jain, Ruchi Jain, Hemant Swami, Sharad Pandey Deepak Kumar Jain. Spectrophotometric method for simultaneous estimation of Simvastatin and Ezetimibe in bulk drug and its combined Dosage form. International Journal of Pharmacy and Pharmaceutical Sciences, **2009,** 1(1), 170-175.

80. J. V. L. N. Seshagiri Rao, D. Anantha Kumar, D. P. Sujan, V. Vijayasree. Simultaneous Determination of Simvastatin and Ezetimibe in Tablets by HPLC**.** E-Journal of Chemistry, **2009,** 6(2), 541-544.

81. Nilesh Jain, Ruchi Jain, Hemant Swami, Deepak Kumar Jain. RP-HPLC method for simultaneous estimation of Simvastatin and Ezetimibe in bulk drug and its combined dosage form**.** Asian J. Research Chem. **2008**, 1(1) 29-31.

82. BG Chaudhari, NM Patel, PB Shah· Determination of Simvastatin, Pravastatin Sodium and Rosuvastatin calcium in tablet dosage forms by HPTLC. IJPS, **2007,** 69(1), 130-132.

83. Vilas. D. Patil, R. Y. Chaudhari. Spectrophotometric method for estimation of Thiocolchicoside and Diclofenac potassium in capsule dosage form by simultaneous equation method. International Journal of Drug Discovery and Herbal Research, (Ijddhr) **2012,** 2(2), 410-412.

84. Bhavin P Marolia, Divyesh J Vanparia, Bhavik H Satani, Pintu B Prajapati, Shailesh A Shah, Dinesh R Shah. Application of RP-HPLC Method for Simultaneous Estimation of Thiocolchicoside and Diclofenac in Commercially Available Capsules. Am. J. Pharm Tech Res, **2012,** 2(3), 806-818.

85. Sunita T. Patil, Vidhya K. Bhusari, Sunil R.Dhaneshwar. Validated HPTLC method for simultaneous estimation of Thiocolchicoside and Aceclofenac in bulk drug and Formulation. International Journal of Pharma and Bio Sciences, **2011,** 2(2), 482- 490**.**

86. Arvind R Umarkar, Niki S Rewatkar, Dynes R Chaple, Lokesh T Thote,Sunil B Chaudhari, Mayur R Bhurat. Stability Indicating RP-HPLC

Method for Estimation of Thiocolchicoside in Capsule Dosage Forms. RJPBCS, **2011**, 2(1) 750-756.

87. Arvind. R. Umarkar, Niki. S. Rewatkar, Manoj. S. Charde, Ritu. M. Charde. Simultaneous Estimation of Thiocolchicoside and Diclofenac Potassium by UV Spectrophotometer using Multi component method. International Journal of Chem Tech Research, **2011,** 3(2), 944-947.

88. Sohan S. Chitlange, Pradeep S. Shinde, Ganesh R. Pawbake, Sagar B. Wankhede. Simultaneous estimation of Thiocolchicoside and Aceclofenac in Pharmaceutical dosage form by spectrophotometric and LC method. Der Pharmacia Lettre, **2010**, 2(2), 86-93.

89. Rachana R Joshi, Krishna R Gupta. Simultaneous UV-Spectrophotometric determination of Thiocolchicoside and Diclofenac in Pharmaceutical formulation. Der Pharmacia Sinica, **2010**, 1 (2), 44-51.

90. Ramchandra Pandey, Pravin O. Patil, Sanjay B. Bari. Validated RP- HPLC Method for Simultaneous Estimation of Thiocolchicoside & Etodolac in Bulk Drug and in Pharmaceutical Dosage Form. Asian Journal of Biochemical and Pharmaceutical Research, **2012**, 2 (1), 381-390.

91. Jyoti Shrivastav, Kamal Shah, Mahadev Mahadik, Sunil R. Dhaneshwar. Application of HPTLC in the simultaneous Estimation of Thiocolchicoside and Diclofenac in bulk drug and pharmaceutical dosage form. Bulletin of Pharmaceutical Research, **2011** 1(3), 34-7.

92. Shekhar M. Bhavsar, Dasharath M. Patel, Amit P. Khandhar, C. N. Patel. Validated RP-HPLC method for simultaneous estimation of Lornoxicam and Thiocolchicoside in solid dosage form. J. Chem. Pharm. Res, **2010**, 2(2), 563-572.

93. Zholt Kormosh, Iryna Hunka, Yaroslavl, Basel. Spectrophotometric determination of Ketoprofen and its application in Pharmaceutical analysis. Anta Poloniae Pharmaceutican Drug Research, **2009**, 66(1), 3- 9.

94. G. Vijayaranga Vittal, R. Deveswaran, S. Bharath, B. V. Basavaraj, V. Madhavan. Development of an Analytical Method for Spectrophotometric Estimation of Ketoprofen using mixed Co Solvency Approach. **2012**, IJPSR, 3(4), 1053-1056.

95. Veena Nair, Mithun S Rajput. A simple spectrophotometric estimation of Ketoprofen in tablets using mixed hydrotropy. Der Pharma Chemica, **2010**, 2(2), 267-271.

96. A. Mohammad, S. Sharma, S.A. Bhawani. Identification of Ketoprofen in Drug Formulation and Spiked Urine Samples by Micelle Thin Layer Chromatography and its Quantitative Estimation by High Performance Liquid Chromatography. International Journal of Pharm Tech Research, **2010**, 2(1), 89-96.

97. B. Tsvetkova, L. Peikova. HPLC Determination of Ketoprofen in Tablet Dosage Forms. Trakia Journal of Sciences, **2013,** 1, 55-59.

98. Rima M. Bankar, Dipti, B. Patel. Simultaneous estimation of Montelukast sodium and Desloratadine by Ratio spectra derivative spectrophotometry method in combined dosage forms. Journal of Chemical and Pharmaceutical Research, **2013** 5(1), 193-199.

99. R.B. Patel, M.R. Patel, J.B. Mehta. Validation of Stability indicating High Performance Liquid chromatographic method for estimation of Desloratadine in tablet formulation. Arabian Journal of Chemistry, **2012**, 3(6).

100. Rele rajan. V, Gurav Pankaj .J. A simple extractive spectrophotometric determination of Loratadine, Desloratadine and Rupatadine from Pharmaceutical formulations. International Journal of Pharma and Bio Sciences, **2012,** 3(2), 89-95.

101. Ekta Sharma, Dr. Nehal J Shah. Development and Validation of First order derivative spectrophotometric method for simultaneous estimation of Ambroxol Hydrochloride and Desloratadine Hydrochloride in combined tablet dosage form. IJPRBS, **2012,** 1(2), 155-156.

102. Satish Bondili, Sudarshan Reddy P. Spectroscopic method for determination of Desolartadine in bulk and its tablet dosage form. **2012,** Int.J. Pharm & Ind. Res, 1(02), 131-134.

103. E.A. Sharma, N. J. Shah. Development and validation of dual wavelength UV spectrophotometric method for simultaneous estimation of Ambroxol Hydrochloride and Desloratadine Hydrochloride in Their Combined Tablet Dosage Form. **2012,** IJPSR, 3(8), 2584-2589.

104. Ekta Sharma, Nehal Shah. Development and Validation of First Order Derivative Spectrophotometric Method for Simultaneous Estimation of Ambroxol and Desloratadine in Combined Tablet Dosage Form. IJPI's Journal of Analytical Chemistry, **2012,** 2(9), 1-7.

105. Sharma Ekta, Shah Nehal J. Development and Validation of High performance thin layer chromatography method for Simultaneous estimation of Ambroxol Hydrochloride and Desloratadine Hydrochloride in their combined tablet dosage form. IRJP, **2012,** 3(5), 305-308.

106. Vibhuti R. Chhatrala, Jitendra Patel. Simultaneous Estimation of Montelukast Sodium and Desloratadine by RP-HPLC in their Marketed

Formulation. International Journal of Chem Tech Research, **2012**, 4(4), 1402 -1407.

107. SV Patel, GF Patel, SG Pipaliya. Development and Validation of Derivative Spectroscopic Method for Simultaneous Estimation of Montelukast Sodium and Desloratadine in Bulk and Combined Dosage Form. Inventi Impact: Pharm Ana & Qual Assur, 2012**,** 1(2), 83-85.

108. Navneet Kumar, Dhanaraj Sangeeth, Pingili Sunil Reddy, Lakkireddy Prakash. A Validated Stability-Indicating RP-UPLC Method for Simultaneous Determination of Desloratadine and Sodium Benzoate in Oral Liquid Pharmaceutical Formulations. Scientia Pharmaceutica, **2012,** 80, 153–165.

109. Umadevi B, Vetrichelvan T. Development and Validation of UV spectrophotometric determination of Doxofylline and Ambroxol HCl in bulk and combined tablet formulation. International journal of current pharmaceutical research, **2011**, 4(6), 1701-1703.

110. Nagavalli D, Abirami.G, Swarna kranti Kumar. Simultaneous estimation of Gemifloxacin Mesylate and Ambroxol hydrochloride in Bulk and tablet dosage form. Journal of Pharmacy research, **2011**, 4(6), 1701-1703.

111. Jain P.S.1. Stability-Indicating HPTLC Determination of Ambroxol Hydrochloride in Bulk Drug and Pharmaceutical Dosage Form. Journal of Chromatographic Science, **2010**, 48(1), 45-48.

112. Mahesh M. Deshpande, Veena S. Kasture, Seema A. Gosavi. Application of HPLC and HPTLC for the simultaneous determination of Cefixime Trihydrate and Ambroxol HCl in pharmaceutical Dosage form. Eurasian Journal of Analytical Chemistry, **2010,** 5(3), 227-238.

113. Senthil Raja M, Shan S.H, Perumal P and Moorthy M.T.S. RP-HPLC method Development and validation for the Simultaneous estimation of Azithromycin and Ambroxol Hydrochloride in tablets. International Journal of Pharmaceutical Technology Research, **2010,** 2(1), 36-39.

114. Makarand Avhad, Dr. Bonde. C. G. Development and Validation of Simultaneous UV spectrophotometric method for the determination of Levofloxacin and Ambroxol in Tablets. International Journal of Chem tech research, **2009** 1(4), 873-888.

115. Krishna Veni Nagappan, Meyyanathan S.N, Rajinikanth B Raja, Suresh Reddy, Jeyaprakash M.R, Arunadevi S Birajdar and Suresh Bhojraj A. RP-HPLC Method for Simultaneous Estimation of Ambroxol Hydrochloride and Loratidine in Pharmaceutical Formulation. Research Journal of Pharm. and Tech, **2008**, 1(4), 366-369.

116. Neela M. Bhatia, Ganbavale S.K, Bhatia M.S, More H.N. and Kokil S.U. RP-HPLC and Spectrophotometric estimation of Ambroxol Hydrochloride and Cetirizine Hydrochloride in combined dosage form. Indian Journal of Pharmaceutical Sciences, **2008**, 70, 603-8.

117. Lakshmana prabu S, Shirwaikar A.A, Dinesh Kumar C. and Aravind Kumar G. Simultaneous UV Spectrophotometric estimation of Ambroxol Hydrochloride and Levocetrizine Dihydrochloride. Indian Journal of Pharmaceutical Sciences, **2008**, 70(2), 236-238.

118. Pai P.N.S, Lalitha N, Balakrishna B and Rao G.K. Determination of Ambroxol Hydrochloride using Dithiocarbamic acid Colorimetric method. Indian Journal of Pharmaceutical Sciences, **2006,** 68(2), 501-2.

119. Meiling Qi, Peng Wang, Ruihua Cong and Jianjun Yang. Simultaneous Determination of Roxithromycin and Ambroxol Hydrochloride in a new tablet formulation by Liquid chromatography. Journal Pharm Biomed Anal, **2004,** 35(5), 1287-91.

120. Dincer zafer, Hasan Basan. and Nil gun Gunden Goger. Quantitative Determination of Ambroxol in tablets by Derivative UV spectrophotometric method and HPLC. Journal of Pharmaceutical and Biomedical Analysis, **2003**, 31(5), 867-872.

121. Kuchekar B. S, Sinde G. S, Naikawadi I. T, Todkark J, Kharade S. V. Spectrophotometric estimation of Ambroxol HCl in tablets. Indian Journal of Pharmaceutical Sciences, **2003,** 25(2), 193-195.

122. Francisco G, Zarzuelo. A, Ma Luisa Sayalero A, Lpez A, Jos. M and Lanao. Determination of Ambroxol Hydrochloride by HPLC. Journal of Liquid Chromatography & Related Technologies, **2001**, 24(7), 1007 – 1014.

123. Narayana reddy M, Kanna roa K. V, Swapna M, Sankar D. G. Spectrophotometric determination of Ambroxol. Indian Journal of Pharmaceutical Sciences, **1998** 60(4), 249-251.

124. Giriraj P, Shajan. A Simultaneous Estimation and Method Validation of Montelukast Sodium and Doxofylline in Solid Dosage form by RP-HPLC. International Journal of Chemical and Pharmaceutical Sciences**, 2011**, 2(1).

125. Akhilesh Gupta, Swati Rawat, Mayuri Gandhi, Jaydeep Singh Yadav. Method Development and Acid Degradation Study of Doxofylline by RP-HPLC and LC-MS/MS. Asian J. Pharm. Ana. **2011,** 1(1), 10-13.

126. Venkatesan S, Giriraj P, Myvizhi S, Kathiravan P and Rameshwar singh. A Simple HPLC Method for Quantitation of Doxofylline in Tablet Dosage

Form. International Journal of Chemical and Pharmaceutical Sciences, **2010,** 1(2), 54-57.

127. HR Joshi, AH Patel, and AD Captain. Spectrophotometric and Reversed-Phase High-Performance Liquid Chromatographic Method for the Determination of Doxophylline in Pharmaceutical Formulations. J Young Pharm, **2010,** 2(3), 289–296.

128. R.Revathi, T.Ethiraj, R.Thenmozhi, V.S.Saravanan, V.Ganesan. High performance liquid chromatographic method development for simultaneous analysis of Doxophylline and Montelukast sodium in a combined form. Pharmaceutical methods, **2011,** 2, 223-228.

129. A. Gupta, V. Yadav, J. S. Yadav, and S. Rawat, An analytical approach of Doxofylline: A Review, Asian Journal of Pharmaceutical Analysis, **2011**, 1(4), 67–70,

130. Maulik Oza, Jagdish Kakadiya, Chirag Oza. Development and Validation of Solvent Extraction Spectrophotometric Method for Simultaneous Estimation of Doxofylline and Terbutaline sulphate in their Combined Dosage Form. American Journal of Pharma tech Research, **2012**, 2 (4), 592-604.

131. Gadara Nirupa. A. Siva Kumar and Upendra M. Tripath. Novel LC Method Development and Validation for Simultaneous Determination of Montelukast and Doxofylline in Bulk and Pharmaceutical dosage form. Journal of Chemistry, **2013**, 10, 7.

132. Atkuru Veera Venkata Naga Krishna Sunil Kumar. Development and Validation of Novel Analytical Methods for Estimation of Doxofylline in Bulk and Dosage Forms**.** European Journal of Chemistry, **2011,** 2(3), 372.

133. Lakshmi Sivasubramanian, V.Sarika, K.Manikandan and K.S.Lakshmi. RP-HPLC and HPTLC Methods for Determination of Doxofylline in Bulk and Formulations. Journal of Pharmacy Research, **2011**, 4(3), 643-644.

134. Amit Kumar De, Ashok Kumar Bera, Biswajit Pal. Development and Validation of Same RP-HPLC Method for Separate Estimation of Theophylline and Doxofylline in Tablet Dosage Forms. Journal of Current Pharmaceutical Research, **2012,** 9 (1), 55-5.

135. Ashu Mittal, Shikha Parmar. Development and Validation of Rapid HPLC Method for Determination of Doxofylline in Bulk Drug and Pharmaceutical Dosage Forms. Journal of Analytical chemistry, 2010, 63, 293-297.

136. Narendra G. Patre, L. Sathiyanarayanan, Mahadeo V. Macadam, Sunil R. Dhaneshwar. A Validated Stability-Indicating HPTLC Method for Analysis of Doxofylline**.** Journal of Planar Chromatography, **2009,** 22(5),345.

137. Mukesh Maithani, Ranjit Singh. Development and Validation of a Stability-Indicating HPLC Method for the Simultaneous Determination of Salbutamol Sulphate and Theophylline in Pharmaceutical Dosage Forms. J Anal Bio anal Techniques**, 2011**, 2, 2-5.

138. Selvadurai Muralidharan, Jay Raj Kumar. High Performance Liquid Chromatographic Method Development and Its Validation for Salbutamol. British Journal of Pharmaceutical Research, **2012**, 2(4), 228-237.

139. Sagar Suman Panda, Bera Venkata Varaha, Ravi Kumar and Ganeshwar Mohanta. Difference UV spectrophotometric method for estimation of levosalbutamol sulphate in tablet dosage form. J Pharm Educ Res, **2012**, 3(1), 17-21.

140. Arun K. Mishra, Manoj Kumar, Amrita Mishra, Anurag Verma, Pronobesh Chattopadhyay. Validated UV spectroscopic method for estimation of Salbutamol from tablet Formulations. Archives of Applied Science Research, **2010,** 2 (3), 207-211.

141. Deepak Kumar Jain, Pratibha Patel, Abhay Kushwaha, Ram Sneh Raghuwanshi, Nilesh Jain. Simultaneous determination of Salbutamol sulphate and Doxophylline in tablets by reverse phase liquid chromatography. Der Pharmacia Lettre, **2011**, 3 (4), 56-62.

142. N Jyothi, K VenuGopal, JVLN Seshagiri Rao. Development and Validation of an HPLC method for the Simultaneous Estimation of the Salbutamol Sulphate and Ipratropium in Inhalation Dosage Forms. International Journal of Pharma Sciences, **2012,** 2(4), 79-83.

143. Pangal Anees. Simple Titrimetric Method for the Estimation of Salbutamol Sulphate (SBS) in Pharmaceutical Formulations. Research Journal of Pharmaceutical Sciences, **2013,** 2(1), 11-14.