METHOD DEVELOPMENT AND VALIDATION OF NEWER ANALYTICAL METHODS AS PER ICH GUIDELINES

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DOCTOR OF PHILOSOPHY
(Faculty of Pharmacy)

Submitted by

Mrs.B. UMADEVI, M.Pharm.

Under the Guidance of
Prof.Dr.T. VETRICHELVAN, M.Pharm., Ph.D.
PRINCIPAL

ADHIPARASAKTHI COLLEGE OF PHARMACY
(Accredited by "NAAC" with CGPA of 2.74 on a four point scale at "B" Grade)
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SEPTEMBER-2013

DECLARATION

I hereby declare that the thesis entitled **"METHOD DEVELOPMENT AND VALIDATION OF NEWER ANALYTICAL METHODS AS PER ICH GUIDELINES"** submitted by me for the degree of DOCTOR OF PHILOSOPHY (Faculty of Pharmacy), is a record of research work carried out by me during the period from 2008-2013, under the guidance of **Prof.Dr.T. VETRICHELVAN, M.Pharm., Ph.D.,** Head, Department of Pharmaceutical Analysis and Principal, Adhiparasakthi College of Pharmacy, Melmaruvathur- 603 319, TamilNadu, India and has not formed the basis for the award of any degree, diploma, associateship, fellowship, title in this or any other university or similar institute of higher learning.

Place: Melmaruvathur **(B. UMADEVI)**

Date: 05.09.2013

CERTIFICATE

This is to certify that the thesis entitled **"METHOD DEVELOPMENT AND VALIDATION OF NEWER ANALYTICAL METHODS AS PER ICH GUIDELINES"** submitted to The TamilNadu Dr. M.G.R. Medical University, Guindy, Chennai- 600 032, Tamil Nadu, India as a partial requirements for the award of Degree of DOCTOR OF PHILOSOPHY (Faculty of Pharmacy) is a record of research work done by **Mrs.B. UMADEVI, M.Pharm.,** during 2008-2013 under my guidance and supervision at Adhiparasakthi College of Pharmacy, Melmaruvathur-603 319, Tamil Nadu, 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: 05.09.2013

Prof.Dr. T.VETRICHELVAN, M.Pharm., Ph.D.,
Head, Department of Pharmaceutical Analysis and Principal,
Adhiparasakthi College of Pharmacy,
Melmaruvathur- 603 319,
Tamil Nadu.

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(UMADEVI. B)

Dedicated to

My

Beloved Parents

and

My Family

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LIST OF ABBREVIATIONS

ICH	-	International Conference on Harmonisation
λ	-	Lambda
LOD	-	Limit of Detection
LOQ	-	Limit of Quantitation
µg/ml	-	Microgram Per Millilitre
ml	-	Millilitre
nm	-	Nanometer
pH	-	Negative Logarithm of Hydrogen Ion
%	-	Percentage
% RSD	-	Percentage Relative Standard Deviation
HPLC	-	High Performance Liquid Chromatography
Rt	-	Retention Time
S.D.	-	Standard Deviation
%RSD	-	Percentage Relative Standard Deviation
S.E.	-	Standard Error
IR	-	Infra Red
°C	-	Degree Celsius
µl	-	Microlitre
min	-	Minute
ml/min	-	Millilitre/minute
HCl	-	Hydrochloric acid
I.P.	-	Indian Pharmacopoeia

AMB	-	Ambroxol
DOX	-	Doxofylline
MET	-	Metolazone
SPIR	-	Spironolactone
METO	-	Metoprolol
OLME	-	Olmesartan
ASP	-	Aspirin
ROSU	-	Rosuvastatin

Introduction

1. INTRODUCTION

1.1 INTRODUCTION TO ANALYTICAL CHEMISTRY[1]

Analytical Chemistry constitutes both theoretical and practical science and it is practical in a large number of laboratories in many different ways. The analytical procedure is the technique of performing the analysis. Analytical method validation is indeed necessary for herbal procedure, new process and reaction, new molecules, active ingredients, residues, impurity profiling and component of interest in different matrices. An analytical methodology comprises of the techniques, method, procedure and protocol. This methodology includes the required data for a given analytical problem, necessary sensitivity, requisite accuracy, mandatory range of analysis and requisite precision to the Analyst.

1.2 WHY VALIDATION ANALYTICAL PROCEDURE[2]

There are many reasons to validate analytical procedures. Among them are regulatory requirements, good science, and quality control requirement. The Code of Federal Regulations (CFR) 311.165c explicitly states that “accuracy, sensitivity, specificity, and reproducibility of test methods employed by the firm shall be established and documented”. Of course as Scientists we would want to apply good science to demonstrate that the analytical method used had demonstrated accuracy, sensitivity, specificity and reproducibility. Finally the management methods had demonstrated uses to release its product are properly validated for its intended use so the product will be safe for human use. Analytical methods need to be validated, verified or revalidated in the following instances.

- use in routine testing

- When transferred to another laboratory
- Whenever the conditions or method parameters for which the method has been validated change.

1.3 PROCESS OF ANALYTICAL METHOD VALIDATION

Process of the analytical method validation is listed below:

1. Preparation of the development on the method validation programme
2. To write the method validation protocol and get the approval
3. Implementation of the method validation protocol
4. Investigation of the method validation data
5. Reporting the analytical method validation
6. Finalizing the analytical method procedure

1.4 ICH GUIDELINES FOR ANALYTICAL METHOD VALIDATION[3,4]

Method validation is the way to authenticate that the analytical procedure applied for a specific test is appropriate for its intended purpose. Methods need to be validated or revalidated. The International Conference of Harmonization (ICH) of technical requirements for the registration of pharmaceutical for human use has developed and provided a consensus text on validation of analytical procedures.

The parameters as defined by the ICH and by other organizations

- ✓ Specificity
- ✓ Selectivity
- ✓ Precision
  - Repeatability
  - Intermediate precision

- Reproducibility

✓ Accuracy

✓ Linearity

✓ Range

✓ Limit of detection

✓ Limit of quantitation

✓ Robustness

✓ Ruggedness

1. 4.1. SPECIFICITY

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

1.4.2. ACCURACY

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

1.4.3. PRECISION

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

Repeatability (Intra- assay precision)

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

Intermediate Precision

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

Reproducibility

Reproducibility measures the precision between laboratories. Reproducibility should be considered in case of the standardization of an analytical procedure, for insistence inclusion of procedure in Pharmacopoeias.

1.4.4. LINEARITY

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

1.4.5. RANGE

Range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample including these concentrations for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

1.4.6. LIMIT OF DETECTION

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

a. Based on visual evaluation

b. Based on signal-to-noise ratio

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

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

1.4.7. LIMIT OF QUANTITATION

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

a. Based on visual evaluation

b. Based on Signal-to- Noise ratio

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

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

1.4.8. ROBUSTNESS

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

1.4.9. RUGGEDNESS

The USP define ruggedness as the degree of reproducibility of test results obtained by the analysis of the same sample under a variety of normal test conditions such as different laboratories, different analysis, different lots of reagents etc. Ruggedness is a measure of reproducibility of test results under normal expected operational conditions from laboratory to laboratory and from Analyst to Analyst.

1.5. SYSTEM SUITABILITY TESTS[5]

System suitability tests are an integral part of Gas and Liquid Chromatography. They are used to verify that the resolution and reproducibility of the chromatographic system and are adequate for the analysis to be done. These tests are based on the concept that the equipment, electronics, analytical operations, samples to be analyzed and constitute an integral system that can be evaluated as such.

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

1.5.1. Capacity Factor (K')

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

$K' = t\text{-}1/t_a$

Where,

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

t_a = retention time of non retarded component, air with thermal conductivity detection.

Limit = generally the value of K' is > 2

1.5.2. Resolution (Rs)

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

$Rs = t_2 - t_1 / 0.5(w_1 - w_2)$

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

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

1.5.3. Tailing Factor (T)

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

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

T = W0.05/ 2f

Where W0.05 = width of peak at 5% height

f = Distance from the peak maximum to the leading edge of the peak, the distance being measured at a point 5% of the peak height from the baseline.

Limit: ≤ 2 is preferable.

1.5.4. Theoretical plates (N)

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

$N = 16(t / w)^2$ or

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

Where

t = retention time of substance.

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

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

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

Limit = N > 2000 is desirable.

1.6 ULTRA VIOLET SPECTROSCOPY[6]

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

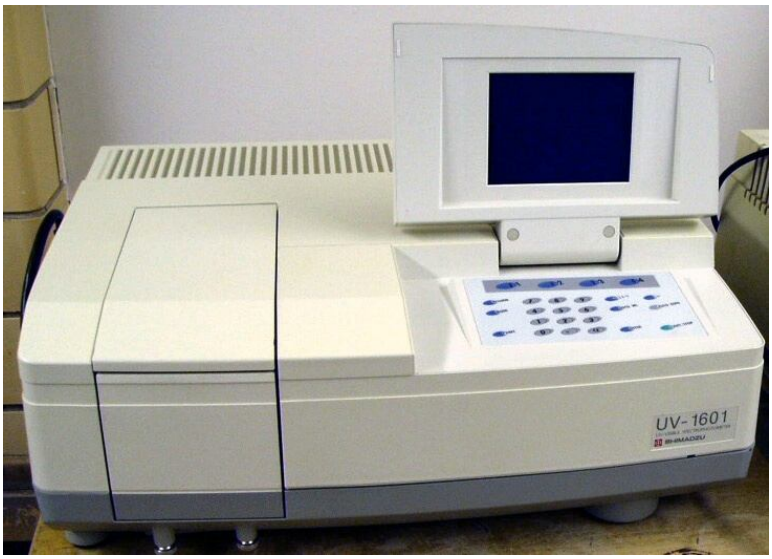
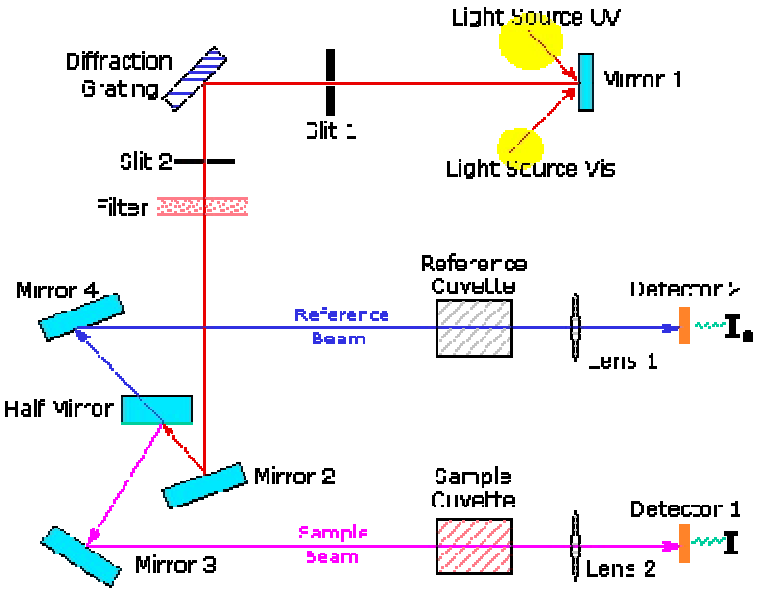


Diagram of UV-Visible Spectrophotometer



Optical Diagram of a Double Beam UV-Visible Spectrophotometer

Quantitative Spectrophotometric Assay of Medicinal Substances

1. Use of a standard absorptivity value

2. Use of a calibration graph

3. Single-or double-point standardization

Methods of Multicomponent Analysis using UV-Visible Spectrophotometer[7]

1. Simultaneous Equation Method

2. Absorbance Ratio or Q-analysis method.

3. Simultaneous equation using area under curve method

4. Derivative Spectroscopy

5. Two-Wavelength method

6. Using multicomponent mode

7. Absorbance Correction Method

8. Geometric Correction Method

9. Orthogonal Polynomial Method

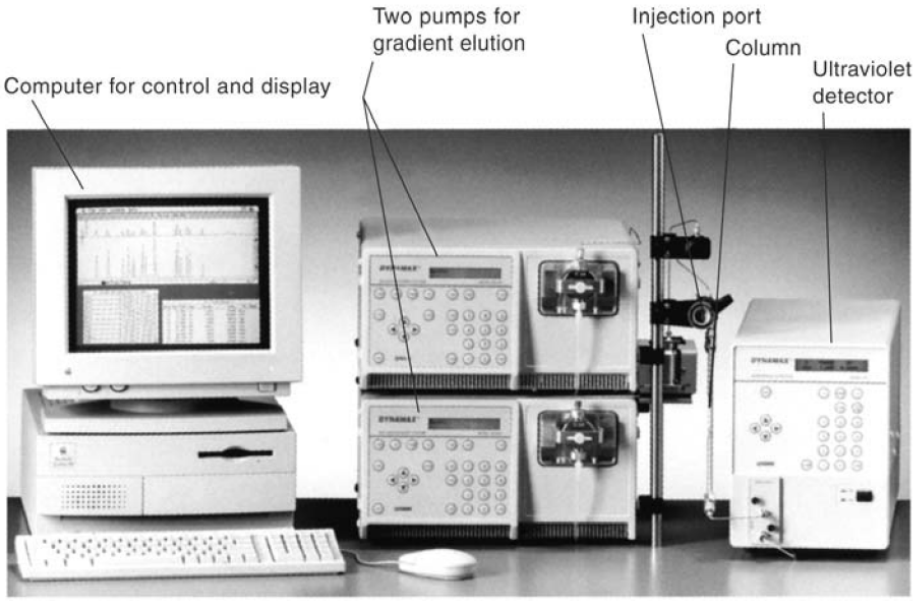
Derivative Spectroscopy

- The UV-Visible spectra consist of increasing or decreasing absorbance as a function of wavelength, A=f(λ): Zero Order
- In derivative Spectroscopy the first or higher derivative of absorbance or transmittance with respect to wavelength is recorded versus the wavelength

1.7 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY[8]

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

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



A Schematic Diagram of HPLC Equipment

1.7.1 Principle of Separation in HPLC[9]

Normal phase chromatography

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

Stationary phase: SiO_2, Al_2O_3, $-NH_2$, -CN, -Diol, $-NO_2$.

Mobile phase: Hectane, Hexane, Cyclohexane, $CHCl_3$, CH_2Cl_2, Dioxane, Methanol.

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

Reverse phase chromatography

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

Stationary Phase: n-octadecyl (RP-18), n-octyl (RP-8), ethyl (RP-2), phenyl, $(CH_2)_n$-CN, $(CH_2)_n$-diol.

Mobile phase: Methanol or Acetonitrile/Water or Buffer (sometimes with additives of THF or Dioxane)

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

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

Aim

&

Objectives

2. AIM AND OBJECTIVES OF THE STUDY

The combined dosage forms selected for the present study are Doxofylline & Ambroxol Hydrochloride, Metolazone & Spironolactone, Metoprolol & Olmesartan Medoxomil and Aspirin & Rosuvastatin in tablets/capsules. These combinations have recently entered into the market.

- Doxofylline & Ambroxol combination is used as an Antiasthmatic agent.
- Metolazone & Spironolactone combination is used as a Diuretic agent.
- Metoprolol & Olmesartan combination is used as an Antihypertensive agent.
- Aspirin & Rosuvastatin combination is used in Cardiovascular diseases.

In the view of the literature cited for the quantification of above mentioned combination of drugs, it was found that the methods for the estimation of Doxofylline, Ambroxol Hydrochloride, Metolazone, Spironolactone, Metoprolol Succinate, Olmesartan Medoxomil, Aspirin & Rosuvastatin in tablets/capsules individually and in combination with other drugs were available. No method available for the simultaneous estimation of the combined dosage forms with the solvents employed for the analytical studies.

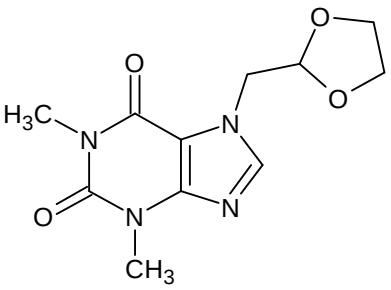
Hence in the present work, the aim is to develop a simple, precise and accurate methods for the estimation of Doxofylline & Ambroxol Hydrochloride, Metolazone & Spironolactone, Metoprolol Succinate & Olmesartan and Aspirin & Rosuvastatin in bulk and in combined Pharmaceutical Dosage form and to validate the developed methods by UV Spectrophotometry, Reverse Phase High-Performance Liquid Chromatography or by both methods.

Review of Literature

3.1 DRUG PROFILE

3.1.1. DOXOFYLLINE[5,10,11,12]

Chemical Structure



Chemical name

7-(1, 3-Dioxolan-2-yl methyl)-3, 7-dihydro-1, 3-dimethyl-1H-Purine-2, 6-Dione.

Molecular formula

$C_{11}H_{14}N_4O_4$

Molecular weight

266.26

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.

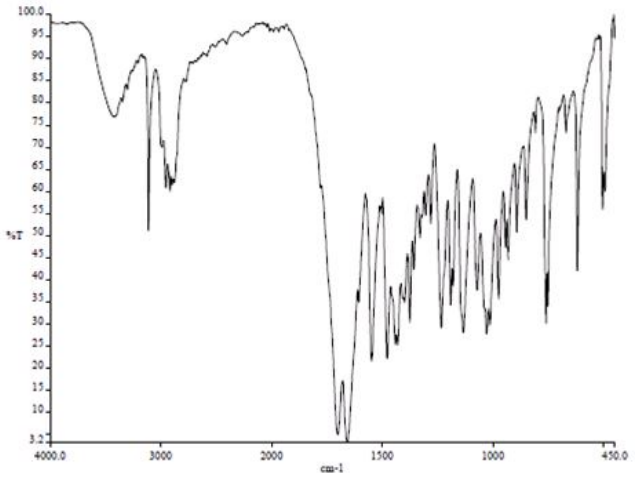
Identification

1. Melting point

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

***Average of six observations**

2. Infra red spectrum



Storage

Store in a cool, dark and dry place

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 bronchodilation 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, extrasystole, 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 upto 1200 mg daily

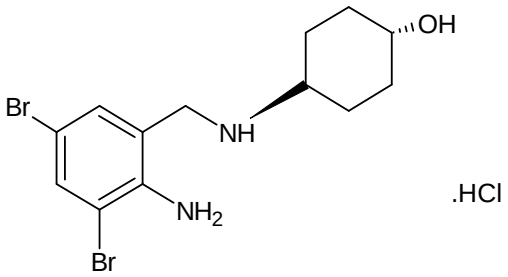
2. It may also be given by slow intravenous injection

Special Precaution:

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

3.1.2 AMBROXOL HYDROCHLORIDE[5,10,11,12]

Chemical Structure



Chemical Name

1 ({[2-Amino-3, 5 dibromo phenyl]-methyl} amino) cyclohexanol monohydrochloride

Molecular formula

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

Molecular weight

414.6

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 6.0

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 ºC -234ºC	233ºC

❖ 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 defense 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.

Overdosage

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

Drug Interactions

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

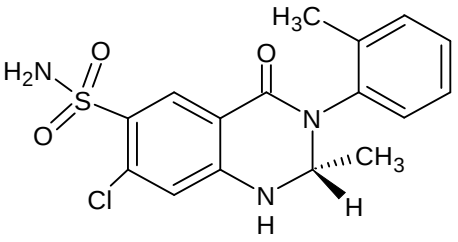
2. No clinically relevant unfavourable interaction with other medications has been reported.

Contraindication

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

3.1.3 METOLAZONE[5,10,11,12]

Chemical Structure



Chemical Name

7-chloro-2-methyl-4-oxo-3-o-tolyl-1,2,3,4-tetrahydroquinazoline-6-sulfonamide

Molecular formula

$C_{16}H_{16}ClN_3O_3S$

Molecular weight

365.84

Category

Antihypertensive agent;

Diuretic

Description

White or slightly yellowish crystalline powder

Solubility

Sparingly soluble in methanol and more soluble in alkali and organic solvents

pka value

12.23

Loss on drying

Maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C for 4 h.

Melting Point

252-254°C

Storage

Preserve in tight, light resistant container

Indication:

Metolazone is indicated for the treatment of hypertension, alone or in combination with other antihypertensive drugs of a different class.

Mode of action:

- Metolazone interferes with the renal tubular mechanism of electrolyte reabsorption.
- It acts primarily to inhibit sodium reabsorption at the cortical diluting site and to a lesser extent in the proximal convoluted tubule. Sodium and chloride ions are excreted in approximately equivalent amounts.

- The increased delivery of sodium to the distal tubular exchange site results in increased potassium excretion. Metolazone does not inhibit carbonic anhydrase.
- The antihypertensive mechanism of action of Metolazone is not fully understood but is presumed to be related to its saluretic and diuretic properties

Pharmacokinetics

Metolazone is slowly and incompletely absorbed from the gastrointestinal tract. An average of about 65% of a dose has been reported to be absorbed after oral administration in healthy subjects, and an average of about 40% in patients with cardiac disease.

About 95% of the drug is bound in the circulation: about 50 to 70% to the red blood cells and between 15 to 33% to plasma proteins. The half-life has been reported to be 8 to 10 hours in whole blood, and 4 to 5 hours in plasma, but the diuretic effect persists for up to 24 hours or more. About 70 to 80% of the amount of Metolazone absorbed is excreted unchanged. The remainder is excreted in the bile and some enterohepatic circulation has been reported. Metolazone crosses the placenta and is distributed into breast milk.

Pharmacodynamics

Metolazone is a quinazoline diuretic, with properties generally similar to the thiazide diuretics. A proximal action of Metolazone has been shown in humans by increased excretion of phosphate and magnesium ions and by a markedly increased fractional excretion of sodium in patients with severely compromised glomerular filtration. This action has been demonstrated in animals by micropuncture studies.

Adverse drug reactions

Palpitation, Chest pain and Chills

Overdosage

Symptoms of overdose include difficulty in breathing, dizziness, dizziness on standing up, drowsiness, fainting, irritation of the stomach and intestines and lethargy leading to coma.

Drug Interactions

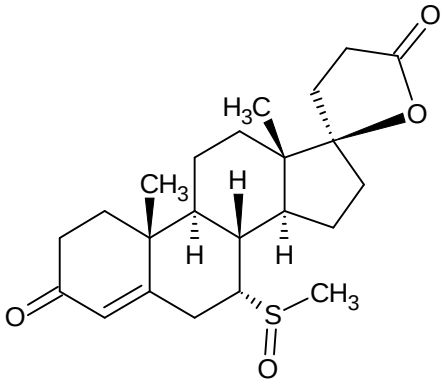
Interacts with digitoxin, digoxin, trandolapril, tenoxicam, etc

Half life

Approximately 14 hours

3.1.4. SPIRONOLACTONE[5,10,11,12,13]

Chemical structure



Chemical Name

7α-acetylthio-3-oxo-17α -pregn-4-ene- 21,17 β -carbolactone

Molecular formula

$C_{24}H_{32}O_4S$

Molecular weight

416.6

Category

- Diuretics

- Aldosterone Antagonists

Description

- Yellowish white to buff coloured powder; Odourless or with a slight odour of thioacetic acid
- It exhibits polymorphism

Solubility

Freely soluble in chloroform; soluble in ethanol (95%); slightly soluble in ether and practically insoluble in water

Loss on drying

Not more than 0.5%, determined on 1 gram by drying in an oven at 105^{o}C for 3 hours

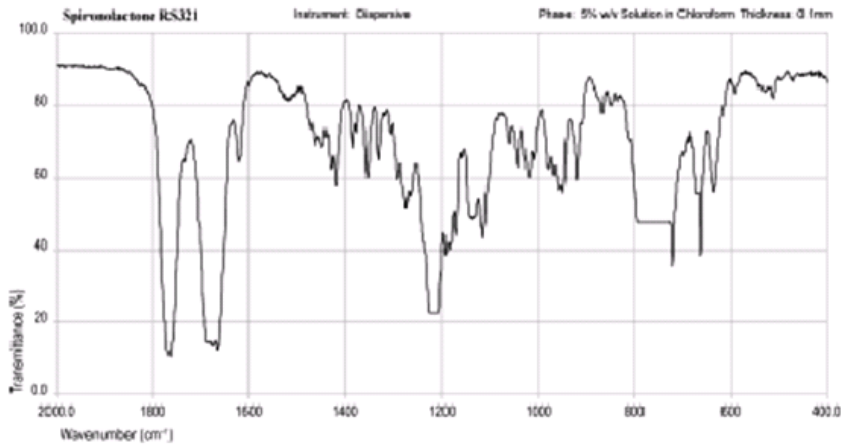
Specific optical rotation

Between -33^{o} and -37^{o}, determined in a 1% w/v solution in chloroform

Identification

1. Melting Point- 134.5°C

2. Infra red spectrum



Storage

Store in well-closed, light-resistant containers

Indication

Spironolactone is primarily indicated to treat low-renin hypertension, hypokalemia, and Conn's syndrome.

Mode of action

Spironolactone is a specific pharmacologic antagonist of aldosterone, acting primarily through competitive binding of receptors at the aldosterone-dependent sodium-potassium exchange site in the distal convoluted renal tubule. Spironolactone causes increased amounts of sodium and water to be excreted, while potassium is retained. Spironolactone acts both as a diuretic and as an antihypertensive drug by this mechanism.

It may be given alone or with other diuretic agents which act more proximally in the renal tubule. Aldosterone interacts with a cytoplasmic mineralocorticoid receptor to enhance the expression of the Na^+, K^+-ATPase and the Na^+ channel involved in a Na^+ K^+ transport in the distal tubule. Spironolactone binds to this mineralcorticoid receptor, blocking the actions of aldosterone on gene expression. Aldosterone is a hormone; its primary function is to retain sodium and excrete potassium in the kidneys.

Pharmacokinetics

Spironolactone is rapidly absorbed from the gastrointestinal tract. Food increases the bioavailability of unmetabolized Spironolactone by almost 100%. Spironolactone and its metabolites are more than 90% bound to plasma proteins. Rapidly and extensively metabolized. The metabolic pathway of Spironolactone is complex and can be divided into two main routes: those in which the sulfur moiety is retained and those in which the sulfur moiety is removed by dethioacetylation. Spironolactone is transformed to a reactive metabolite that can inactivate adrenal and testicular cytochrome P450 enzymes.

It also has anti-androgenic activity. The metabolites are excreted primarily in the urine and secondarily in bile.

Overdosage:

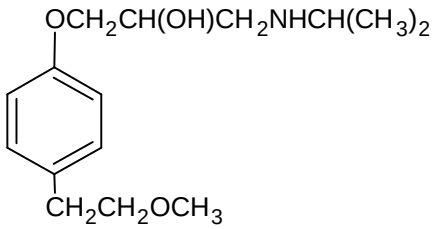
The oral LD_{50} of Spironolactone is greater than 1,000 mg/kg in mice, rats, and rabbits. Acute overdosage of Spironolactone may be manifested by drowsiness, mental confusion, maculopapular or erythematous rash, nausea, vomiting, dizziness, or diarrhea. Spironolactone has been shown to be a tumorigen in chronic toxicity studies in rat.

Half life:

10 minutes

3.1.5. METOPROLOL[5,10,11,12]

Chemical Structure



Chemical name

±-1-(Isopropylamino)-3-[p-(2-methoxyethyl) phenoxy]-2-propanol

Molecular formula

$C_{15}H_{25}NO_3$

Molecular weight

652.81

Categories

- Antihypertensive agent
- Adrenergic agent

- Adrenergic beta-Antagonists
- Sympatholytics
- Antiarrhythmic Agents

Description

White or almost white crystalline powder.

Solubility

Freely soluble in water, soluble in methanol, slightly soluble in alcohol, very slightly soluble in ethyl acetate.

pH

A 6.5% solution in water has a pH between 7.0 and 7.6

Loss on drying

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

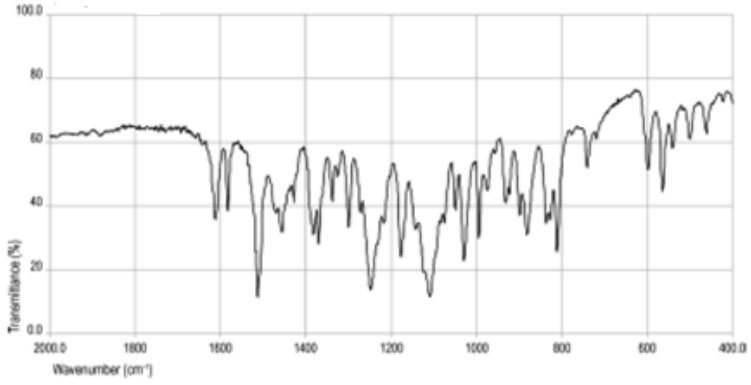
Assay

Dissolve 0.250 g in 40 ml of anhydrous acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically

Identification

1. Melting Point- 136°-137°C

2. Infra Red Spectrum



Storage

Store in air tight containers at controlled room temperature. Protect from light

Indication

Metoprolol is indicated for the management of acute myocardial infarction, angina pectoris, heart failure and mild to moderate hypertension. May be used to treat supraventricular and tachyarrhythmias and as prophylaxis for migraine headaches.

Mode of action

Metoprolol competes with adrenergic neurotransmitters such as catecholamines for binding at beta (1)-adrenergic receptors in the heart. Beta (1)-receptor blockade results in a decrease in heart rate, cardiac output, and blood pressure.

Pharmacokinetics

- Metoprolol is readily and completely absorbed from the gastrointestinal tract but is subject to considerable first-pass metabolism, with a bioavailability of about 50%.
- Peak plasma concentration varies widely and occurs about 1.5-2 hours after a single oral dose. It is moderately lipid-soluble.
- Metoprolol is widely distributed; it crosses the blood-brain barrier and the placenta, and is distributed into breast milk. It is about 12% bound to plasma protein.
- It is extensively metabolized in the liver, predominantly by the cytochrome P450 isoenzyme CYP2D6, and undergoes oxidative deamination, and aliphatic hydroxylation.
- The metabolites are excreted in the urine together with only small amounts of unchanged Metoprolol. The rate of metabolism by CYP2D6 is determined by genetic polymorphism.

Pharmacodynamics

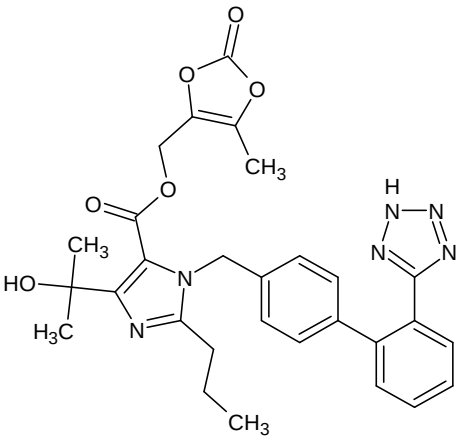
Metoprolol, a competitive, beta1-selective (cardioselective) adrenergic antagonist, is similar to Atenolol in its moderate lipid solubility, lack of intrinsic sympathomimetic activity and weak membrane stabilizing activity.

Toxicity:

LD_{50}=5500 mg/kg (orally in rats), toxic effects include bradycardia, hypotension, bronchospasm, and cardiac failure. LD_{50}=2090 mg/kg (orally in mice)

3.1.6 OLMESARTAN MEDOXOMIL[10,12,14]

Chemical Structure



Chemical Name

(5-methyl-2-oxo-2H-1,3-dioxol-4-yl)methyl 4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)-1H-imidazole-5-carboxylate

Molecular formula

$C_{29}H_{30}N_6O_6$

Molecular Weight

558.585

Category

- Antihypertensive Agents
- Angiotensin II Type 1 Receptor Blockers

Description

White to off-white crystalline powder

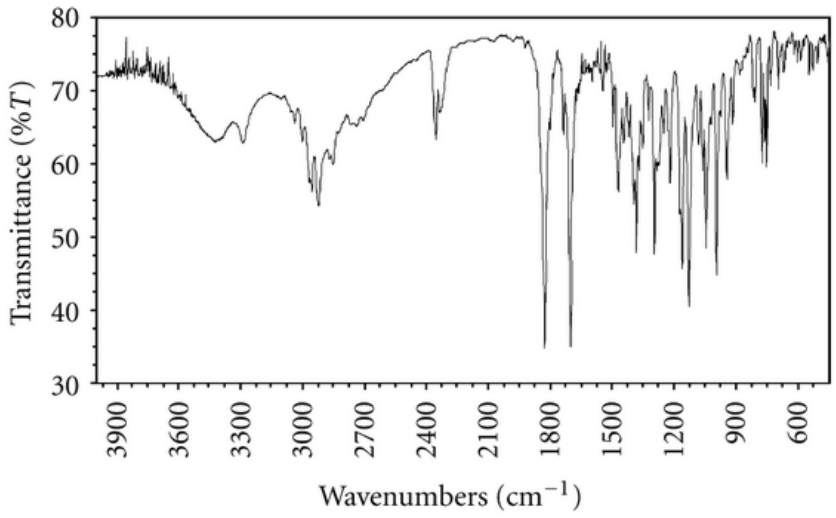
Solubility

Insoluble in water; Sparingly soluble in strong acid; Soluble in strong base.

Identification

1. Melting Point: Standard value 175-180°C

2. IR spectrum



Storage

Store in a tightly closed container in a dry place

Indication

Olmesartan Medoximil is indicated for the treatment of Hypertension

Mode of action

Angiotensin II is formed from angiotensin I in a reaction catalyzed by angiotensin converting enzyme (ACE, kininase II). Angiotensin II is the principal pressor agent of the renin-angiotensin system, with effects that include vasoconstriction, stimulation of synthesis and release of aldosterone, cardiac stimulation and renal reabsorption of sodium. Olmesartan blocks the vasoconstrictor effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in vascular smooth muscle. Its action is, therefore, independent of the pathways for angiotensin II synthesis. Olmesartan has more than a 12,500-fold greater affinity for the AT1 receptor than for the AT2 receptor.

Pharmacokinetics

- Olmesartan medoxomil is an ester prodrug that is hydrolysed during absorption from the gastrointestinal tract to the active form Olmesartan. The absolute bioavailability is approximately 26%.
- Peak plasma concentrations of Olmesartan occur about 1 to 2 hours after oral administration.
- Volume of distribution is about 17 L. Highly bound to plasma proteins (99%) and does not penetrate red blood cells.
- It is excreted in the urine and the bile as Olmesartan; about 35 to 50% of the absorbed dose is excreted in the urine and the remainder in the bile. The terminal elimination half-life is between 10 to 15 hours.

Pharmacodynamics

Olmesartan, a specific angiotensin II type 1 antagonist, is used alone or with other antihypertensive agents to treat hypertension. Unlike the angiotensin receptor antagonist Losartan, Olmesartan does not have an active metabolite or possess uricosuric effects. Blockade of the angiotensin II receptor inhibits the negative regulatory feedback of angiotensin II on renin secretion, but the resulting increased plasma renin activity and circulating angiotensin II levels do not overcome the effect of Olmesartan on blood pressure.

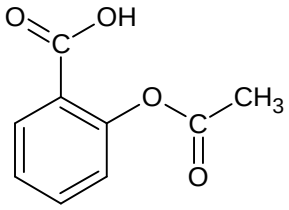
Toxicity

Symptoms of overdose include dehydration (dry mouth, excessive thirst, muscle pain or cramps, nausea and vomiting, weakness), dizziness, low blood pressure, and slow or irregular heartbeat.

3.1.7. ASPIRIN[10,12,13]

Acetyl Salicylic Acid

Chemical Structure



Chemical Name

2-acetoxy benzoic acid

Molecular Formula

$C_9H_8O_4$

Molecular Weight

180.2

Category

- Antiplatelet
- Analgesic
- Antipyretic

Description

Colourless crystals or a white crystalline powder; Odourless or almost odourless

Solubility

Slightly Soluble in water; freely soluble in alcohol; and sparingly soluble in absolute ether

Loss on drying

Not more than 0.5%, determined on 1.0 g by drying over phosphorus pentoxide at a pressure of 1.5 to 2.5 kPa

Assay

Weigh accurately about 1.5 g, dissolve in 15 ml of ethanol (95%), add 50.0 ml of 0.5 M sodium hydroxide, boil gently for 10 minutes, cool and titrate the excess of alkali with 0.5 M HCl using phenol red solution as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of sodium hydroxide required.

Melting Point

Standard value-135°C

Storage

Store protected from moisture at a temperature not exceeding 30°C

Indication

- Aspirin is indicated for the mild to moderate pain such as Headache, Dysmenorrhoea, Myalgia, and Dental pain
- And also indicated in the initial treatment of Angina Pectoris, Myocardial infarction and for the prevention of cerebrovascular disorders such as Stroke.
- In the management of pain and inflammation in acute and chronic rheumatoid disorders such as Rheumatoid arthritis, Juvenile idiopathic arthritis, Osteoarthritis, and Ankylosing spondylitis.

Mode of action

It acts as the inhibitors of the enzyme cyclo-oxygenase, which results in the direct inhibition of the biosynthesis of Prostaglandins and thrombaxanes from arachidonic acid. Aspirin also inhibits platelet aggregation: non-acetylated salicylates do not.

Pharmacokinetics

1. Absorption

Aspirin and other salicylates are absorbed rapidly from the gastrointestinal tract when taken orally but absorption following rectal administration is less reliable. Aspirin and other salicylates can also be absorbed through the skin. After oral doses, absorption of non-ionised aspirin occurs in the stomach and intestine. Some aspirin is hydrolysed to salicylate in the gut wall

2. Distribution

Aspirin is 80 to 90% bound to plasma proteins and is widely distributed. Its volume of distribution is reported to be 170 ml/kg in adults. As plasma drug concentration increase, the binding sites on the protein becomes saturated and V_d increases.

3. Metabolism

Salicylates are mainly eliminated by hepatic metabolism. The metabolites include salicyluric acid, salicyl phenolic glucoronide, salicyl acyl glucoronide, gentisic acid and gentisuric acid

4. Excretion

Following oral administration, elimination is a first-order process and the plasma-salicylate half life is about 2 to 3 hours. At high Aspirin doses, the half life increases to 15 to 30 hours. Salicyalte is also excreted unchanged in urine, the amount excreted by this route increases with increasing dose and also depends on urinary pH. Renal excretion involves glomerular filtration, active renal tubular secretion and passive tubular reabsorption

Adverse drug reaction

- The most common adverse effects of therapeutic doses of aspirin are gastrointestinal disturbances such as nausea, dyspepsia, and vomiting.
- Salicylism characterized by tinnitus, vertigo, decreased hearing and sometimes also nausea and vomiting occurs with overdosage of any salicylate.
- Reye's syndrome, a rare disorder of children that is characterized by hepatic encephalopathy following an acute viral illness and 20-40% mortality.
- Salicylate poisoning is a result of disturbances of the acid-base and electrolyte balance in patients with high doses of salicylate-containing drug.
- Large doses can cause depression of the respiratory centre.
- In the CNS, initial stimulation with excitement is followed eventually by coma and respiratory depression.

- Disturbances of haemostasis as a result of depressed platelet aggregation

Overdosage

High doses may precipitate acute haemolytic anaemia in patients with G6PD deficiency. Aspirin may interfere with insulin and glucagon control in diabetes.

Drug interaction

- Aspirin causes potentially hazardous increase in the effect of Warfarin
- Aspirin interferes with the uricusoric agents such as Probenecid and Sulfinpyrazone.
- Use of Aspirin with Dipyridamole may result in an increase in plasma-salicylate concentration.
- Drugs such as Metoclopramide in patients with migraine headache result in earlier absorption of aspirin and higher peak plasma concentration.
- Metoprolol may increase peak plasma-salicylate concentrations.

Contraindication

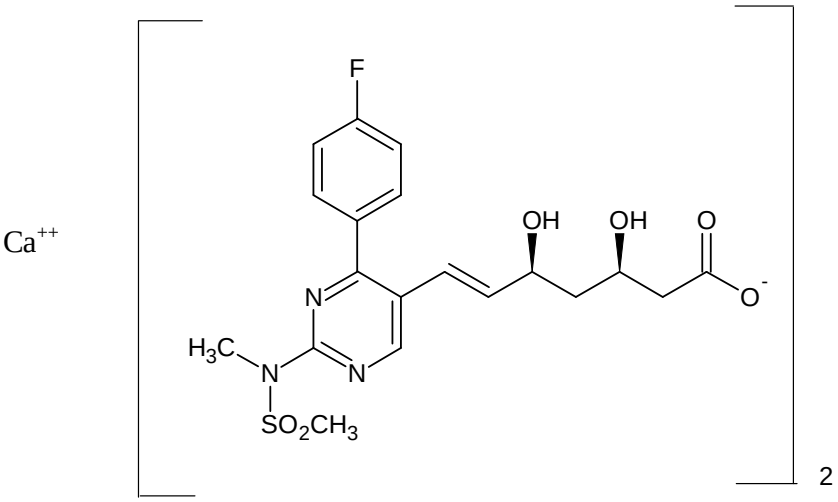
It is contraindicated in patients prone to dyspepsia or known to have the lesion of the gastric mucosa. It should not be given to patients with haemophilia or other haemorrhagic disorders.

Precaution

Aspirin should be used with caution in patients with asthma or allergic disorders. It should not be given to patients with a history of sensitivity reactions to aspirin or other NSAIDs, including those in whom attacks of asthma, angioedema, urticaria, or rhinitis have been precipitated by such drugs.

3.1.8. Rosuvastatin Calcium[10,12,13]

Chemical Structure:



Chemical name

(3R,5S,6E)-7-[4-(4-flurophenyl)-2-(N-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5 dihydroxyhept-6-enoic acid calcium

Molecular Formula

$(C_{22}H_{27}FN_3O_6S)_2$ Ca

Molecular Weight

1001.1

Category

- Anticholesteremic Agents
- HMG-CoA Reductase Inhibitors

Description

An off-white to creamish white crystalline powder

Solubility

Sparingly soluble in water and methanol; slightly soluble in ethanol

Identification

1. Melting point: Std value-155°C

2. IR Spectrum: IR spectrum of Rosuvastatin Calcium is compared with the standard values; principal peaks at a wave numbers were identified.

Storage

Store protected from light and moisture

Indication

- Used as an adjunct to dietary therapy to treat primary hypercholesterolemia (heterozygous familial and nonfamilial), mixed dyslipidemia and hypertriglyceridemia.
- Also indicated for homozygous familial hypercholesterolemia as an adjunct to other lipid-lowering therapies or when other such therapies are not available.

Mode of action

Rosuvastatin is a competitive inhibitor of HMG-CoA reductase. HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonate, an early rate-limiting step in cholesterol biosynthesis. Rosuvastatin acts primarily in the liver. Decreased hepatic cholesterol concentrations stimulate the upregulation of hepatic low density lipoprotein (LDL) receptors which increases hepatic uptake of LDL. Rosuvastatin also inhibits hepatic synthesis of very low density lipoprotein (VLDL). The overall effect is a decrease in plasma LDL and VLDL

In vitro and in vivo animal studies also demonstrate that rosuvastatin exerts vasculoprotective effects independent of its lipid-lowering properties.

Pharmacokinetics

1. Absorption

Rosuvastatin is incompletely absorbed from the gastrointestinal tract, with a bioavailability of about 20%. Peak plasma concentrations are achieved about 5 hours after an oral dose.

2. Distribution

Rosuvastatin is 90% bound to plasma proteins mostly albumin. This binding is reversible and independent of plasma concentration. Volume of distribution is 134 L.

3. Metabolism

It is taken extensively by the liver, its primary site of action, and undergoes limited metabolism, mainly by the cytochrome P450 CYP2C9.

4. Excretion

The plasma elimination half life of Rosuvastatin is about 19 hours. Approximately 90% of an oral dose of Rosuvastatin is excreted in the faeces, including absorbed and non-absorbed drug, and the remainder is excreted in the urine; about 5% of a dose is excreted unchanged in urine.

Pharmacodynamics

Rosuvastatin is a synthetic, enantiomerically pure antilipemic agent. It is used to lower total cholesterol, LDL-Cholesterol, apolipoprotein B (apoB), non-HDL-Cholesterol, and triglyceride (TG) plasma concentrations while increasing HDL-C concentrations. High LDL-C, low HDL-C and high TG concentrations in the plasma are associated with increased risk of atherosclerosis and cardiovascular disease. The total cholesterol to HDL-C ratio is a strong predictor of coronary artery disease and high ratios are associated

with higher risk of disease. Increased levels of HDL-C are associated with lower cardiovascular risk. By decreasing LDL-C and TG and increasing HDL-C, rosuvastatin reduces the risk of cardiovascular morbidity and mortality.

Adverse drug reactions

Generally well-tolerated. Side effects may include myalgia, constipation, asthenia, abdominal pain, and nausea. Other possible side effects include myotoxicity (myopathy, myositis, rhabdomyolysis) and hepatotoxicity.

Drug Interactions

The risk of skeletal muscle effects may be enhanced when Rosuvastatin is used in combination with niacin.

Contraindications

Patients with a known hypersensitivity to any component of this product. Hypersensitivity reactions including rash, prutritis, urticaria and angioedema.

Precaution

Caution is advised when using this drug in the elderly because they may be more sensitive to its side effects, especially muscle injury.

3.2 REPORTED METHODS

3.2.1. REPORTED METHODS FOR DOXOFYLLINE:

Franco Tagliaro *et al.*[15] **(1990),** reported **"Non-Extraction HPLC Method for Simultaneous Measurement of Dyphylline and Doxofylline in Serum"**. This HPLC method is based on direct injection mode for the simultaneous measurement of Dyphylline and Doxofylline in serum. Chromatographic separation was performed on "Pinkerton" internal surface reversed-phase column and phosphate buffer (0.1 mol/l, pH 6.8) as mobile phase and monitored at 275 nm.

Xu Yanggui *et al.*[16] (**2002**), reported "**Determination of Doxofylline Concentration in Plasma by RP-HPLC**". The mobile phase was composed of methanol-water-triethylamine-acetic acid glacial (35: 65: 0.01: 0.01) at a flow rate of 1 ml/min and the detecting wavelength was 272 nm. All data were dealt with by "3p97" program. The method is found to be rapid, accurate and precise and it is suitable for clinical pharmacokinetic study.

Wang Shujun *et al.*[17] (**2003**), reported "**Determination of Doxofylline Concentration in Human Serum by HPLC**". A Techsphere ODS colum was used with a mobile phase of methanol: water (312: 488) at a flow rate of 0.8 ml/min and detecting wavelength was 273 nm. Serum sample was used for chromatography after protein precipitation and centrifuging.

Guo Junping *et al.*[18] **(2005)**, reported "**Determination of Doxofylline in Plasma by SPE-HPLC**". The Doxofylline concentrations in plasma through solid-phase extraction were determined by HPLC method. RSD of intraday and interday were less than 10%

accuracy accorded with the plasma concentration monitoring and pharmacokinetic study. The SPE-HPLC method to determine Doxofylline concentration is an ideal method.

Lagana A *et al.*[19] **(2005),** reported "**Solid Phase Extraction and High Performance Liquid Chromatog raphic Determination of Doxofylline in Plasma".** The developed sensitive and selective High Performance Liquid Chromatographic Doxofylline assay used ultraviolet detection for plasma samples. The drug is isolated from biological samples with a reversed phase C_{18} disposable extraction column. Plasma standard curves are linear for concentrations of Doxofylline from 0.03 to 10 mg/L.

Xie Zi-li[20] (**2006**), reported **"Determination of Doxofylline in Doxofylline Injection by GC Method".** The capillary column used was DM-17 (30 mm X 0.32 mm X 0.25 μm). The carrier gas was nitrogen, and the detector was FID. The column temperature was 265°C and Papaverine Hydrochloride was selected as internal standard. The assay of Doxofylline was calculated by internal standard method.

Gannu R *et al.*[21] **(2007),** reported **"Development and Validation of a Stability-Indicating RP-HPLC Method for Analysis of Doxofylline in Human Serum**. **Application of the Method to a Pharmacokinetic Study".** The developed method was carried out by precipitation for isolation and sample concentration, followed by Reverse-Phase Liquid chromatographic analysis at 275 nm. The chromatographic separation was performed on C_{18} column with samples containing the internal standard (Metronidazole) and Doxofylline. The mobile phase used is 18:82 acetonitrile-phosphate buffer (12.5 mM potassium dihydrogen orthophosphate, pH 3.0) at a flow rate of 1 ml/min. The validated method was applied to a pharmacokinetic study of Doxofylline in human serum after administration of a single Doxofylline tablet (400 mg).

Kamila MM *et al.*[22] **(2007),** reported "**Development and Validation of Spectrophotometric Method for Estimation of Anti-Asthmatic Drug Doxofylline in Bulk and Pharmaceutical Formulation"**. The developed method utilized Double beam UV-Visible Spectrophotometer (UV-2450, Shimadzu, Japan) and has showed absorption maximum at 272 nm in water.

Sreenivas N *et al.*[23] (2008), **reported** "Development and Validation of a Sensitive LC-MS/MS Method with Electrospray Ionization for Quantitation of Doxofylline in Human Serum: Application to a Clinical Pharmacokinetic Study". **The developed and validated LC-MS/MS method for the estimation of Doxofylline with 300 µL human serum using Imipramine as the internal standard. The API-3,000 LC-MS/MS was operated under multiple reaction-monitoring modes using the electrospray ionization technique. The assay procedure involved direct precipitation of Doxofylline and Internal Standard from human serum with acetonitrile. The resolution of peaks was achieved with formic acid (pH 2.5): acetonitrile (10: 90) on an Amazon C_{18} column.**

Anurekha Jain *et al.*[24] **(2009)** reported, "**Analytical Method Development, Validation and Comparison of Spectrophotometric and Stability Indicating HPLC Methods for the Simultaneous Estimation of Doxofylline and Montelukast in Pharmaceutical Dosage Form**". Two methods were developed. The first method is RP-HPLC. The separation was carried out by Phenyl Inertsil column (250 mm X 4.6 mm) at a flow rate of 2 ml/min. The compounds were eluted using mobile phase of ammonium acetate (0.05 M) at pH 3.5 mixed by a low pressure gradient program with methanol and detected at 274 nm. The second method is First Order Derivative spectroscopy at zero crossing over

Wavelengths selected were 255.5 nm and 369 nm for Doxofylline and Montelukast respectively.

Kan Quan-cheng *et al.*[25] **(2009)**, reported, "**HPLC Determination of Doxofylline and Pharmacokinetic Study in Serum of Patients with Chronic Obstructive Pulmonary Disease".** The Doxofylline in serum after intravenous injection was determinated by HPLC at 273 nm. The sample was separated on Waters C_{18} column (150 mm X 3.9 mm, 4 μm) with mobile phase consisting of 0.1% triethylamine- 0.02 mol/L NaH_2PO_4 buffer (pH 6.8 ± 0.1) (15: 85) at a flow rate 1.0 ml/min. The pharmacokinetic parameters were analyzed by 3P97.

Narendra G. Patre *et al.*[26] **(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.

Ashu Mittal *et al.*[27] **(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.

Joshi HR *et al.*[28] **(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.

Liu Yifang *et al.*[29] (**2010**), reported **"Determination of Theophylline and Doxofylline in Human Plasma by HPLC"**. The developed method used C_{18} column with caffeine as internal standard and the mobile phase of methanol-water (23:77). The detection wavelength was 273 nm. The plasma samples were injected directly after precipitation by methanol. The calibration curves of Theophylline and Doxofylline were linear in the concentration range of 2.5-40 μg/ml. The intra-and interday RSDs were less than 5.2%.

Venkatesan S *et al.*[30] (**2010**) reported "**A Simple HPLC Method for Quantitation of Doxofylline in Tablet Dosage Form**". The developed Reverse-Phase High Performance Liquid chromatographic method used inertsil octyl decyl column in isocratic mode with mobile phase consisting of Methanol: Water (30:70) at a flow rate of 1.5 ml/min. The eluents were monitored at 274 nm.

Akhilesh G *et al.*[31] **(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.

Atkuru Veera Venkata Naga Krishna Sunil Kumar *et al*.[32] (**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.

Giriraj P and Shajan A[33] (**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.

Lakshmi Sivasubramanian *et al.*[34] **(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.

Revathi R *et al*.[35] (**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.

Gadapa Nirupa *et al.*[36] (**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 C_{18} 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.

Maulik Oza *et al.*[37] (**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.

3.2.2. REPORTED METHODS OF AMBROXOL HYDROCHLORIDE:

Francisco G *et al.*[38] **(2001)**, reported "**Determination of Ambroxol Hydrochloride by HPLC**". Reverse-Phase High Performance Liquid Chromatography was employed, using methanol: 0.01M diammonium phosphate buffer of pH 6, in the ratio of 70:30 and monitored at 247 nm**.**

Dincer Z *et al.*[39] **(2003),** reported "**Quantitative Determination of Ambroxol in Tablets by Derivative UV Spectrophotometric Method and HPLC**". The Ambroxol was determined by First-order derivative UV-spectrophotometric method at 255 nm. The chromatographic method was performed on C_{18} column with a mixture of aqueous phosphate (0.01 M), acetonitrile and glacial acetic acid in the ratio of 59:40:1.

Meiling Qi *et al.*[40] **(2004),** reported "**Simultaneous Determination of Roxithromycin and Ambroxol Hydrochloride in a New Tablet Formulation by Liquid Chromatography**". The chromatographic method was carried out on a Diamonsil TM C_{18} column. The mobile phase comprised of a mixture of acetonitrile, methanol and 0.5% ammonium acetate (39:11:50). Detection was carried out at 220nm.

Pai PNS *et al.*[41] **(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 coloured copper chelate. The yellowish-orange chromophore has absorption maxima at 448 nm.

Kothekar KM *et al.*[42] **(2007),** reported **"Quantitative Determination of Levofloxacin and Ambroxol Hydrochloride in Pharmaceutical Dosage Form by Reversed-Phase High Performance Liquid Chromatography"**. Chromatographic separation was performed on Hypersil BDS C_{18} column (25 cm X 4.6 mm, 5 µm). The mobile phase consisted of Buffer: Acetonitrile: Methanol (650: 250: 100) with triethylamine and pH adjusted to 5.2 with dilute phosphoric acid at flow rate of 1.0 ml/min and monitored at 220 nm.

Bhatia NM *et al.*[43] **(2008)**, reported "**RP-HPLC and Spectrophotometric Estimation of Ambroxol Hydrochloride and Cetirizine Hydrochloride in Combined Dosage Form**". The chromatographic methods were standardized using a HIQ SIL-C_{18} column (250 X 4.6 mm i.d., 10 µm) with UV detection at 229 nm and mobile phase consisting of methanol-acetonitrile-water (40:40:20).

Lakshmana Prabhu S *et al.*[44] **(2008)**, reported **"Simultaneous UV Spectrophotometric Estimation of Ambroxol Hydrochloride and Levocetirizine Dihydrochloride".** The developed method was found to be simple, accurate and reproducible. A Shimadzu UV/Visible spectrophotometer, model 1601 was used in this method and the measurement of absorbance at 242 and 231 nm for Ambroxol Hydrochloride and Levocetirizine Dihydrochloride respectively.

Krishna Veni Nagappan *et al.*[45] **(2008)**, reported "**A RP-HPLC Method for Simultaneous Estimation of Ambroxol Hydrochloride and Loratidine in Pharmaceutical Formulation".** The developed Reverse Phase HPLC method is simple, selective, rapid, precise and economical. This method utilized Phenomenex Gemini C_{18} (25 cm X 4.6 mm i.d., 5 µ) column with a mobile phase comprised of acetonitrile: 50mM Ammonium Acetate (50:50) at a flow rate of 1.0 ml/min, with detection at 255 nm.

Deshpande MM *et al.*[46] **(2010)** reported **"Application of HPLC and HPTLC for the Simultaneous Determination of Cefixime Trihydrate and Ambroxol Hydrochloride in Pharmaceutical Dosage Form".** The methods employed are 1. High Performance Thin Layer Chromatography and 2. High Performance Liquid Chromatography. In HPTLC followed by densitometric measurements, the spots were made at 254 nm. The chromatographic separation was carried out on HPTLC aluminium sheets of silica gel 60

F254 and mobile phase containing acetonitrile: methanol: triethylamine in the ratio of 8.2:1:0.8. In HPLC method, the chromatographic separation was made on C_{18} column using mobile phase as acetonitrile: methanol in the ratio of 50:50.

Dhiraj S. Nikam *et al.*[47] **(2010)** reported **"Stability Indicating RP-HPLC Method for Simultaneous Estimation of Ambroxol Hydrochloride and Roxithromycin in Bulk and Tablet Dosage Form".** The chromatographic separation was made on Phenomenex Gemini C_{18} column (250 cm X 4.6 mm, 5 µm) and mobile phase consisted of water: acetonitrile: orthophosphoric acid (50:50:0.1), at a flow rate of 1 ml/min and monitored at 210 nm.

Jain PSI[48] **(2010)**, reported "**Stability-Indicating HPTLC determination of Ambroxol Hydrochloride in bulk drug and pharmaceutical dosage form**". The Chromatography separation was carried out on HPTLC aluminium plates precoated with silica gel 60F-254 and the solvent system consisted of methanol-triethylamine (4:6). Densitometric analysis of Ambroxol Hydrochloride was carried out in the absorbance mode at 254 nm.

Prashanthi NL *et al.*[49] **(2010),** reported **"Estimation of Ambroxol Hydrochloride and Guiaphensin in Tablet Dosage Form by Simultaneous Equation Method"**. The absorbance for Ambroxol Hydrochloride and Guiaphensin were measured at 242 and 272 nm respectively. Beer's law was obeyed at the concentration range of 5-50 µg/ml for Ambroxol and 10-80 µg/ml for Guiaphensin. The molar absorptivity for Ambroxol and Guiaphensin were 9742 ± 0.894 and 1015 ± 0.707 respectively.

Trivedi Aditya and Banerjee Lopamudra[50] **(2010),** reported **"Development of Modified Spectrophotometric and HPLC method for Simultaneous Estimation of Ambroxol Hydrochloride and Cetirizine Hydrochloride in Tablet Dosage Form".** UV-visible

spectroscopy utilized water as a solvent at 20°C for the estimation of drugs. The developed RP-HPLC was carried on a C_{18} column with mobile phase consisted of acetonitrile, methanol and water in the ratio of 10:20:70, at 1 ml/min and detected at 244 and 230 nm for Ambroxol Hydrochloride and Cetirizine Hydrochloride respectively.

Maithani M *et al.*[51] **(2010),** reported "**Simultaneous estimation of Ambroxol Hydrochloride and Cetirizine Hydrochloride in Tablet Dosage Form by RP-HPLC Method".** The developed Reverse Phase High Performance Liquid Chromatographic method is simple, specific and accurate. The chromatographic separation was made on Princeton C-8 (4.6 X 25 mm, 5 µm) column and mobile phase comprised of methanol and potassium dihydrogen phosphate buffer in the ratio of 80:20 adjusted to pH 3.5 ± 0.02 with orthophosphoric acid, at a flow rate of 1.0 ml/min and were measured at 276 nm.

Senthil Raja M *et al.*[52] (**2010),** reported **"RP-HPLC Method Development and Validation for the Simultaneous Estimation of Azithromycin and Ambroxol Hydrochloride in Tablets".** The chromatographic separation was carried out using mobile phase consisting of acetonitrile and mono basic potassium phosphate buffer of pH 8.5 in the ratio of 65:35. The column used was C_{18} phenomenex Gemini 5m, 250cm X 4.6mm id with flow rate of 2 ml/min using PDA detection at 220 nm.

Prathap B *et al.*[53] **(2010),** reported "**Simultaneous Determination of Gatifloxacin and Ambroxol Hydrochloride from Tablet Dosage Form using RP-HPLC".** The developed and validated Reversed-Phase High Performance Liquid Chromatography method was used for the quantitative determination of Gatifloxacin and Ambroxol Hydrochloride, from its tablet dosage form. Chromatographic separation was made on a

Thermo Hypersil Keystone ODS C_{18} column (250 mm X 4.6 mm, 5 μm), with a mobile phase consisting of a mixture of phosphate buffer and acetonitrile (60:40), and pH adjusted to 3 with orthophosphoric acid, at a flow rate of 1 ml/min and monitored at 250 nm.

Ilangovan Ponnilavarasan *et al.*[54] **(2011)**, reported **"Simultaneous Estimation of Ambroxol Hydrochloride and Loratadine in Tablet Dosage Form by using UV Spectrophotometric Method".** The spectrophotometric method developed is rapid, simple, accurate, sensitive and specific. The absorbance for Ambroxol and Loratadine was measured at 308 nm and 245 nm respectively.

Trivedi RK *et al.*[55] **(2011),** reported **"A Rapid, Stability Indicating RP-UPLC Method for Simultaneous Determination of Ambroxol Hydrochloride, Cetirizine Hydrochloride and Antimicrobial Preservatives in Liquid Pharmaceutical Formulation".** The developed and validated RP-UPLC method is selective, precise, accurate, linear, filter compatible and robust. The chromatographic separation was carried out on Agilent Eclipse plus C_{18} (50 mm X 2.1 mm, 1.8 μm) column using gradient elution at 237 nm detector wavelength. The mobile phase consisted of a mixture of 0.01 M phosphate buffer in 0.1% triethylamine for Solvent-A and acetonitrile for Solvent B.

Prathap B *et al.*[56] **(2011),** reported **"Spectrophotometric Method for Simultaneous Estimation of Gatifloxacin and Ambroxol Hydrochloride in Tablet Dosage Form".** This method utilized Shimadzu UV-1700 using solvent system of methanol and 0.1 M Sodium hydroxide in the ratio of 8:2.

Wankhede SB *et al.*[57] **(2011),** reported **"Simultaneous Spectrophotometric Estimation of Gemifloxacin Mesylate and Ambroxol Hydrochloride in Tablets".** The developed two UV-spectrometric methods were simple, sensitive and rapid. In the simultaneous equation method, the absorbance of Gemifloxacin and Ambroxol were measured at 272 and 249.5 nm respectively. In the first order derivative spectroscopy method, wavelengths selected for quantitation were 216 nm for Gemifloxacin and 279 nm for Ambroxol.

Patel PA *et al.*[58] **(2011),** reported **"Spectrophotometric Simultaneous Estimation of Salbutamol and Ambroxol in Bulk and Formulation"**. The two developed methods are 1. Simultaneous equation method and 2. Area under the curve method. In simultaneous equation method, the measurement of absorbance was made at 223 nm and 244 nm for Salbutamol sulphate and Ambroxol Hydrochloride respectively. In area under the curve method, the wavelength range was 232-217 nm for Salbutamol and 252-237 nm for Ambroxol.

Patel PA *et al.*[59] **(2011)** reported **"Simultaneous Determination of Salbutamol and Ambroxol in Fixed Dose Combination by Spectrophotometry"**. The methods developed are 1. Absorbance correction method and 2. First order derivative method. In both the methods linearity was found to be in the concentration range of 2-10 µg/ml and 2-20 µg/ml for Salbutamol and Ambroxol respectively and correlation co-efficient was found to be around 0.998. The percent recovery for Salbutamol and Ambroxol was found to be in the range of 98.20 to 102%.

Nagavalli D *et al.*[60] **(2011),** reported **"Validated HPLC Method for the Simultaneous Estimation of Gemifloxacin Mesylate and Ambroxol Hydrochloride in Bulk and**

Tablet Dosage Form". The developed RP-HPLC method used Phenomenex C_{18} column with Acetonitrile: Methanol: 0.1% Trifluroacetic acid (25:20:55) as mobile phase, at 1 ml/min and monitored at 248 nm.

Dhaneshwar SR *et al.*[61] **(2011),** reported **"Validated HPTLC Method for Simultaneous Estimation of Amoxycillin Trihydrate and Ambroxol Hydrochloride in Pharmaceutical Dosage Form".** Chromatographic separation was made on aluminium plates precoated with silica gel 60 F254 and solvent system consisted of N-butanol: 1.0 M Ammonium acetate: Methanol in the ratio of 7.5:2.0:1.5. Densitometric evaluation of the separated zone was performed at 222 nm.

Avinash V. Deosarkar *et al.*[62] (**2012**), reported "**Simultaneous Quantification of Salbutamol Sulphate and Ambroxol Hydrochloride by RP-HPLC and HPTLC in Bulk Drug and Dosage Form**". Two methods were developed 1.Reverse Phase High Performance Liquid Chromatography and 2. High Performance Thin Layer Chromatography. In the RP-HPLC method, Inertsil, ODS-3V C_{18} (250 mm X 4.6 mm, 5μm) column in isocratic mode was used with mobile phase comprising of acetonitrile: 50 mM disodium hydrogen phosphate buffer (containing 0.1% triethylamine, pH 4.2) (28:72) at a flow rate of 1mL/min. In the HPTLC method, the chromatograms were developed using a mobile phase of methanol: ethyl acetate: toluene: ammonia (4:1.5:5.6:1.0) on precoated plate of silica gel 60 F_{254} and quantified by densitometric absorbance mode at 231 nm.

Ekta Sharma and Dr. Nehal J Shah[63] (**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". A double beam UV/Visible spectrophotometer was used. Ambroxol Hydrochloride and Desloratadine hydrochloride showed significant first derivative absorbance at 256 nm (Zero crossing point of Desloratadine Hydrochloride) and 308 nm (Zero crossing point of Ambroxol Hydrochloride) respectively.

Gopalakrishnan S *et al.*[64] (**2012**), reported "**Development of RP-HPLC Method for the Simultaneous Estimation of Ambroxol Hydrochloride, Cetirizine Hydrochloride and Antimicrobial Preservatives in Combined Dosage Form**". The developed RP-HPLC method for simultaneous determination of Ambroxol Hydrochloride, Cetirizine hydrochloride, Methylparaben and Propylparaben in combined liquid pharmaceutical formulation was carried out on Hypersil BDS C_{18} (200 mm X 4.6mm, 5µm) column using acetonitrile: 0.05 M potassium dihydrogen orthophosphate, pH 3.5 (33:67) at a flow rate of 1 ml/min and effluent was detected at 230 nm.

Jigar Goswami *et al.*[65] (**2012**), reported "**RP- HPLC Method Development and Validation for Simultaneous Estimation of Ambroxol Hydrochloride and Cefpodoxime Proxetile in Pharmaceutical Dosage Form**". The Chromatographic analysis was performed on Phenomenex Luna C_{18} column with mobile phase containing Acetonitrile: 0.05 M Potassium Dihydrogen Ortho Phosphate Buffer (70:30), pH 6.7 at a flow rate of 1.0 ml/min and detected at 245 nm.

Madhura V. Dhoka and Shakuntala S. Chopade[66] (**2012**), reported "**Method Development & Comparative Statistical Evaluation of HPLC & HPTLC Method for Simultaneous Estimation of Cefodrixil Monohydrate & Ambroxol Hydrochloride**". Two methods were developed 1. High Performance Liquid Chromatography and 2. High Performance Thin Layer Chromatography. The HPLC method was standardised using

Purospher BDS C_{18} column (25 cm X 4.6mm, 5µm) with mobile phase consisting of 0.5M ammonium acetate buffer- acetonitirile (50:50), pH 7 at a flow rate of 1.0 ml/min and detected at 247 nm. HPTLC analysis was carried out on precoated TLC plates, coated with silica gel 60 F_{254} with mobile phase consisting of methanol-potassium dihydrogen phosphate (0.067 M) (35:65) and scanned at 254nm.

Nidhi Dubey *et al.*[67] (**2012**), reported "**Development of HPLC Method for Simultaneous Estimation of Ambroxol, Guaifenesin and Salbutamol in Single Dose Form".** The chromotographic separation was achieved on C_8 column (250 mm X 4.6 mm, 5 µm) in isocratic mode with mobile phase consisting of disodium hydrogen orthophosphate buffer: methanol, pH 4.5 at a flow rate of 1 ml/min. Scanning was performed at 220 nm.

Rakesh Kotkar P *et al.*[68] (**2012**), reported "**Development and Validation of RP-HPLC Method for Simultaneous Estimation of Cefpodoxime Proxetil and Ambroxol Hydrochloride in Bulk and in Tablets**". RP-HPLC method was carried out on a Qualisil RP C_8 (250 mm X 4.6 mm, 5 µm) column with a mobile phase consisting of acetonitrile: 0.025 M potassium dihydrogen phosphate buffer (70:30), pH 4.0 at a flow rate of 1.0 ml/min. The eluents were detected at 248 nm using Diclofenac sodium as an internal standard.

Ramalingam Suresh *et al.*[69] (**2012**) reported "**HPLC Method for the Simultaneous Determination of Levocetirizine, Ambroxol and Montelukast in Human Plasma Employing Response Surface Methodology**". The chromatographic analysis was performed on Phenomenex C_{18} analytical column (150 mm X 4.6 mm, 5µm) with mobile phase consisting of MeOH-MeCN-dipotassium hydrogen phosphate buffer (pH 7.0)

(32.7:30: 37.3) at a flow rate of 0.85 ml/min. The eluate was monitored using an UV detector set at 230 nm.

Rele Rajan V and Gurav Pankaj J[70] (**2012**), reported "**Simple Spectrophotometric Methods for Determination of Ambroxol Hydrochloride from Pharmaceutical Formulation**". A Shimadzu-UV 1800 double beam UV-visible Spectrophotometer was used. The drug was diazotised with sodium nitrite in presence of acetic acid and coupled with catechol or resorcinol or β-naphthol in alkaline medium. The resulting coloured chromogenic species in solution were directly measured at 425 nm.

Sharma EA and Shah NJ[71] (**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**". 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.

Sharma Ekta A and Shah Nehal J[72] (**2012**), reported "**Development and Validation of High Performance Thin Layer Chromatography for Simultaneous Estimation of Ambroxol Hydrochloride and Desloratadine Hydrochloride in their Combined Tablet Dosage Form**". The chromatographic analysis was carried out on aluminium plates precoated with silica gel 60 F254 with the solvent system consisting of Chloroform: Ethyl acetate: Methanol: Triethylamine (6: 4.5: 2.5: 0.8). Densitometric evaluation was performed at 254 nm.

3.2.3. REPORTED METHODS OF METOLAZONE:

Brodie RR *et al.*[73] (**1981**), reported **"Determination of the Diuretic Agent Metolazone in Plasma by High-Performance Liquid Chromatography".** The chromotographic analysis for the quantification of Metolazone in plasma was performed on C_{18} column (30 cm X 0.4 cm, 10 µm) with mobile phase consisting of 46% (v/v) acetonitrile in aqueous potassium dihydrogen orthophosphate (0.l %w/v) at a flow rate of 2 ml/min.

Vose CW *et al.*[74] **(1981)**, reported **"Quantitation of Metolazone in Plasma and Urine by High Performance Liquid Chromatography with Fluorescence Detection"**. The chromatography was performed on SAS-Hypersil C_8-alkyl silylated silica column (10 cm X 5 mm, 5 µm) with methanol: water (35:65) as mobile phase at a flow rate of 2.3 ml/min using 2-isopropyl analogue as an internal standard, and detected by fluorescence spectra.

Don Farthing *et al.*[75] (**1990**), reported **"Quantitation of Metolazone in Urine by High-Performance Liquid Chromatography with Fluorescence Detection".** The chromatographic separation was performed on Nucleosil C_{l8} column (1.5 cm X 4.4 mm I.D, 5 µm) and mobile consisting of monobasic potassium phosphate-acetonitrile (65:35), pH 3.0 at a flow rate of 1 ml/min and detected by fluorescence.

Don Farthing *et al.*[76] (**1994**), reported "**Novel High-Performance Liquid Chromatographic Method Using Solid-Phase On-Line Elution for Determination of Metolazone in Plasma and Whole Blood**". High Performance Liquid Chromotographic method was developed on Spherisorb ODS C_{18} column (10 cm x 4.6 mm I.D, 3 µm) with mobile phase consisting of monobasic potassium phosphate-acetonitrile (70:30), pH 3.0

at a flow rate of 1 ml/min. On-line elution and fluorescence detection were utilized in this method.

Guangli Wei *et al*.[77] **(2007)**, reported "**Determination of Metolazone in Human Blood by Liquid Chromatography with Electrospray Ionization Tandem Mass Spectrometry**". The developed Liquid Chromatography with Electrospray Ionization Tandem Mass Spectrometry was performed on Diamonsil C_{18} column (200 mm X 4.6 mm, 5μm) and mobile phase comprising of acetonitrile, 10 mmol/l ammonium acetate and formic acid in the ratio of 60:40:0.1, at a flow rate of 0.5 ml/min. Electrospray ionization source was operated in positive ion mode.

Shikha M. N. Roy *et al*.[78] **(2008)**, reported **"LC-MS-MS Method for Determination of Metolazone in Human Plasma"**. High Performance Liquid Chromatography coupled to Electrospray Tandem Mass Spectrometry (LC-MS-MS) method for the quantification of Metolazone in human plasma was performed on C_{18} column (50 mm X 4.6 mm i.d, 5 μm) with 2 mM ammonium acetate buffer, pH 3.0 and acetonitrile (20:80) as mobile phase at a flow rate of 0.4 ml/min under isocratic condition.

Jadhav V *et al.*[79] **(2009),** reported **"Validation of Reverse Phase High Performance Liquid Chromatography Method of Metolazone and Its Determination in Bulk Drug and Pharmaceutical Dosage Form".** JASCO HPLC system 2000 series instrument was used with Thermo C_{18} column (250 X 4.6 mm, 5 μm) and the mobile phase consisting of acetonitrile: water in the ratio of 50:50, at a flow rate of 1 ml/min. Detection wavelength used is 236 nm.

Ramkumar Dubey *et al*.[80] **(2011)**, reported **"Validated RP-HPLC Method for Simultaneous Quantitation of Losartan Potassium and Metolazone in Bulk Drug**

and Formulation". The developed and validated Reverse-Phase High Performance Liquid Chromatography method was carried out on a Thermo Hypersil BDS-C_{18} column (250 mm X 4.6 mm, 5.0 μm) with mobile phase comprising of acetonitrile:water (60:40) at a flow rate of 0.8 ml/min, under isocratic condition using UV detection at 237 nm.

Ramkumar Dubey *et al.*[81] (**2011**), reported "**Validated HPTLC Method for Simultaneous Estimation of Losartan potassium and Metolazone in Bulk Drug and Formulation**". High Performance Thin Layer Chromotographic analysis was performed on silica gel precoated aluminum plate 60 F-254 plates [20 cm X 10 cm 250 μm thickness] with mobile phase consisting of toluene : ethyl acetate : methanol : glacial acetic acid (6 : 4 : 1 : 0.1) and Densitometric measurement was made at 237 nm.

Sandeep Kumar S *et al.*[82] **(2011),** reported **"Development and Validation of Visible Spectrophotometric Methods for the Estimation of Metolazone in Pharmaceutical Dosage Forms"**. Shimadzu 1800 UV-Visible spectrophotometer was used. Two methods are developed. First method is based on oxidative coupling reaction of Metolazone with MBTH to produce green colored chromogen and second method is based on reaction of Metolazone with Folin-Ciocalteu reagent in alkaline media to produce blue coloured chromogen. The absorption maximum was obtained at 623 nm and 725 nm for first and second methods respectively.

Shobha Manjunath *et al.*[83] (**2011**) reported "**Ultra Violet and Derivative Spectrophotometric Methods for Estimation of Metolazone in Pharmaceuticals**". Double beam Shimadzu 1700 UV-Visible Spectrophotometer was used. Metolazone produced maximum absorption at 237nm and 270 nm in water (Method A) and alcohol

(Method B) respectively. In first order derivative spectra (Method C) a sharp peak is obtained at 229.6nm in water.

Devika GS *et al.*[84] (**2012**), reported "**RP-HPLC Method for Simultaneous Estimation of Metolazone and Ramipril in Oral Solid Dosage Form**". The developed and validated HPLC method was performed on Hypersil C_{18} column (250 mm X 4.6 mm, 50 μm) with mobile phase composed of 30 mM sodium dihydrogen phosphate buffer, pH 3: acetonitrile in the ratio of 40: 60, at a flow rate of 1.0 ml/min, and detected at 242 nm.

Durga Prasad B *et al.*[85] **(2012)**, reported **"A Validated UV Spectroscopic Method of Metolazone in Bulk and its Tablet Dosage Forms"**. Shimadzu 1700 U.V. visible double beam spectrophotometer with 1cm U.V. matched quartz cells were used. The developed and validated spectrophotometric method in 0.1 N NaOH showed maximum absorption at 236.5 nm.

3.2.4. REPORTED METHODS OF SPIRONOLACTONE:

Chamberlain J[86] (**1971**), reported "**Gas Chromatographic Determination of Levels of Aldadiene in Human Plasma and Urine Following Therapeutic Doses of Spironolactone**". The developed method involves the extraction of the metabolite from the plasma or urine into dichloroethane with subsequent separation and detection on a gas chromatograph. A Pye 104 chromatograph was used with silanised glass columns (1.5 m long x 4 mm I.D.) and equipped with a single ^{63}Ni electron-capture detector.

Rosa Herraez-Hernandez *et al.*[87] (**1994**) reported "**High-Performance Liquid Chromatographic Determination of Spironolactone and its Major Metabolite Canrenone in Urine using Ultraviolet Detection and Column-Switching**". The

developed High Performance Liquid Chromatographic method involving a column-switching system utilized Hypersil ODS-C_{18} (20 mm X 2.1 mm, 30 μm) pre-column and the analytical separation was carried on LiChrospher RP C_{18} column (125 mm x 4 mm, 5 μm) with mobile phase consisting of acetonitrile-water in gradient elution mode at a flow rate of 1 ml/min. The chromatographic signal for Spironolactone and Canrenone was detected at 230 nm and 300 nm respectively.

Parimoo P *et al.*[88] (**1995**), reported "**Simultaneous Determinations of Spironolactone with Hydroflumethiazide and Spironolactone with Frusemide in Combination Formulations by UV Absorption Method**". In this Simultaneous analysis method, λmax of Spironolactone, Hydroflumethiazide and Frusemide were found to be 238 nm, 273 nm and 276 nm respectively, without any interference from each drug.

Erdal Dinc and Ozgur Ustundag[89] (**2003**), reported "**Spectophotometric Quantitative Resolution of Hydrochlorothiazide and Spironolactone in Tablets by Chemometric Analysis Methods**". Shimadzu UV-1600 double beam UV- Visible spectrophotometer was used. Four Chemometric analysis methods were developed using zero-order and first derivative spectra 1. Classical least-squares, 2. Inverse least-squares, 3. Principal component regression and 4. Partial least-squares. Hydrochlorothiazide exhibits two absorption maxima at 226.4 and 270.6 nm, while Spironolactone gives an absorption maximum at 240.4 nm.

Ebru Tekerek *et al.*[90] **(2008)**, reported "**Quantitative Determination of Hydrochlorothiazide and Spironolactone in Tablets by Spectrophotometric and HPLC Methods**". Two methods were developed 1.Spectroscopic method (Vierodt method & Absorbance Ratio Method) and 2. HPLC method. In the absorbance ratio

method, 260 nm was selected as the isosbestic point. For Vierordt method, $A_1^{\ 1}$ values were calculated at 242 nm and 269 nm for Hydrochlorothiazide and Spironolactone respectively. The developed High Performance Liquid Chromatographic analysis was performed isocratically on Luna.C_{18} (250 mm X 2.6 mm, 5 μm) reversed phase column with mobile phase consisting of water-methanol-phosphate buffer (pH 3.0 ± 0.1) in the ratio of 71:25:4, and the eluent was monitored at 240 nm.

Hiresh K. Golher *et al*.[91] (**2010**), reported "**Simultaneous Spectrophotometric Estimation of Torsemide and Spironolactone in Tablet Dosage Form**". The developed three spectrophotometric methods were 1. Absorbance ratio, 2. First order derivative spectroscopy method and 3. Area under curve (AUC) method. The double beam UV/Visible spectrophotometer (Shimadzu-1601) was used with methanol as a solvent. Torsemide and Spironolactone showed maximum absorbance at 288 nm and 238 nm respectively.

Irena Baranowska *et al*.[92] (**2010**), reported, "**Rapid UHPLC Method for Simultaneous Determination of Vancomycin, Terbinafine, Spironolactone, Furosemide and Their Metabolites: Application to Human Plasma and Urine**". The Ultra High Performance Liquid Chromatography - UV method utilized reversed-phase Hypersil GOLD C_{18}e column (50 mm X 2.1 mm, 1.7 μm) with mobile phase consisted of acetonitrile - 0.1% formic acid in gradient elution mode. The elutes were monitored by UV detection at 280 nm, 224 nm, 280 nm, 224 nm, 215 nm, 245 nm and 280 nm for Saluamine, N-desmethyl carboxy terfinafine, Furosemide, Terbinafine, Vancomycin, Spironolactone and Canrenone in human plasma and urine respectively.

Smita Sharma *et al.*[93] **(2010)**, reported **"Conventional and Micellar Liquid Chromatography Method with Validation of Torsemide and Spironolactone in Tablet Combined Dosage Form"**. The developed and validated Micellar Liquid Chromatographic method was performed on Licrosphere C_{18} column (250 X 4.6 mm) using Tween-20, N-butanol phosphate buffer (50:25:25) adjusted to pH 3.5 ± 0.01, at a flow rate of 1.5 ml/min and UV detection at 254 nm.

Smita Sharma *et al.*[94] (**2010**), reported **"Isocratic Reverse Phase HPLC Estimation Method of Torsemide and Spironolactone in Pharmaceutical Combined Dosage Form"**. The developed and validated HPLC method for estimation of Torsemide and Spironolactone in tablet dosage form was achieved on Licrosphere C_{18} column (250mm X 4.6mm) using Methanol: Acetonitrile: Phosphate buffer, pH 6.5 (60:20:20) at a flow rate of 1.5 ml/min in an isocratic mode, and UV detection at 252 nm.

Sharma MC *et al.*[95] (**2010**), reported "**Validated TLC Densitometric method for the quantification of Torsemide and Spironolactone in bulk drug and in tablet dosage form**". The developed and validated High Performance Thin Liquid Chromatographic (HPTLC) method used precoated silica gel 50 F_{254} with mobile phase consisting of a mixture of ethyl acetate: acetone: acetic acid (10.5: 4: 1.5), and the spot was detected at 269 nm.

Yin-Hai Ma *et al.*[96] **(2010),** reported **"Determination of Atenolol, Rosuvastatin, Spironolactone, Glibenclamide and Naproxen in Blood by Rapid Analysis Liquid Chromatography".** The rapid High Performance Liquid Chromatographic method used ZORBAX stable bound (4.6 mm X 50 mm, 1.8 µm) C18 rapid analysis column with

mobile phase consisting of methanol and 0.01 mol/L of acetic acid in the ratio of 78:22, at a flow rate of 2.0 ml/min and detected at 235 nm.

Maha A. Hegazy *et al.*[97] (**2011**), reported "**Validated Chromatographic Methods for Determination of Hydrochlorothiazide and Spironolactone in Pharmaceutical Formulation in Presence of Impurities and Degradants**". The developed two chromatographic methods were 1. Thin Layer Chromatography-Densitometry and 2. Reverse Phase High-Performance Thin Layer Chromatography. In TLC-densitometric method, the chromatographic separation was made on TLC aluminum sheets (20 X 10 cm) precoated with 0.25 mm silica gel 60 F_{254} with mobile phase consisting of ethyl acetate-chloroform-formic acid-triethyl amine (7:3:0.1:0.1) and scanning was performed on Camage TLC scanner 3 at 235 nm. In HPLC method, RP-C_{18} column (220 mm X 4.6mm, 5 μm) with mobile phase consisting of water-acetonitrile (97:3) as the initial proportion, and the gradient elution was used during the analysis (from 97:3 to 65:35), at a flow rate of 2 ml/min. The effluent was detected at 230 nm.

Bhojani Maulik *et al.*[98] (**2012**), reported "**Development and Validation of RP-HPLC Method for Simultaneous Estimation of Furosemide and Spironolactone in their Combined Tablet Dosage Form**". The chromatographic analysis was performed on Inertsil C_{18} column (250mm X 4.6mm, 5μm) with methanol-water in the ratio of (70:30) as mobile phase, pH 3.20 ± 0.05 at a flow rate of 1 ml/min. The detection wavelength was 236 nm.

Chandrakanth Vadloori and Venkat Tallada[99] (**2012**), reported "**Development and Validation of RP-HPLC Method for Simultaneous Estimation of Spironolactone and Frusemide in Bulk and Pharmaceutical Dosage Forms".** High Performance Liquid

Chromatography separation was made on Azilent Zobax Rx C_8 column (4.6 mm X 150 mm, 5 µm) with mobile phase comprising of potassium dihydrogen phosphate buffer (pH 7.51) and methanol in the ratio of 60:40, at a flow rate of 1.0 ml/min, and detected at 215 nm.

Hardik Patel and Sagar Solanki[100] (**2012**), reported "**Development and Validation of Spectrophotometric Methods for Simultaneous Estimation of Furosemide and Spironolactone in Combined Tablet Dosage Form**". The developed two spectrophotometric methods were 1. First order Derivative spectroscopy and 2. Absorbance Ratio (Q-Absorbance) method. An UV-Visible double beam spectrophotometer (Shimadzu 1800) was used. Methanol was chosen as a solvent. In the first order derivative spectra, Furosemide and Spironolactone were measured at amplitudes of 350 nm and 250.80 nm at the zero crossing point of Spironolactone and Furosemide respectively. The wavelength ranges 261.21 nm (iso-absorptive point) and 276 nm (λmax of Furosemide) were selected for Absorbance ratio method.

3.2.5. REPORTED METHODS OF METOPROLOL

Aqil M *et al.*[101] **(2007),** reported **"A Validated HPLC Method for Estimation of Metoprolol in Human Plasma".** The chromatographic separation was made on C_{18} column with acetonitrile: water: triethylamine in the ratio of 18:81:1 as mobile phase and pinacidil monohydrate as internal standard. The UV detection was made at 275 nm.

Sarkar AK *et al.*[102] **(2008),** reported **"Simultaneous Determination of Metoprolol Succinate and Amlodipine Besylate in Human Plasma by Liquid Chromatography-Tandem Mass Spectrometry Method and Its Application in Bioequivalence Study".**The developed and validated method for quantification of Metoprolol succinate

and Amlodipine Besylate in human plasma used hydrochlorothiazide as internal standard. The chromatographic analysis was performed on a Reversed-Phase peerless basic C18 column with a mobile phase of methanol-water containing 0.5% formic acid (8:2). The protonated analyte was quantitated in positive ionization by multiple reaction monitoring with a mass spectrometer.

Venkateswarlu P *et al.*[103] **(2010),** reported **"Selective and Sensitive Method for the Determination of Metoprolol in Human Plasma using Liquid Chromatography Coupled with Tandem Mass Spectrometry".** The developed and validated High-Performance Liquid Chromatography-Tandem Mass spectrometric method was performed on C_{18} analytical column in isocratic mode with mobile phase consisting of consisting of 10 mmol/L ammonium acetate, pH 5.0/acetonitrile (15:85) at a flow rate of 1 ml/min. The atmospheric pressure chemical ionization technique was used for sample ionization in positive ion mode and enhanced selectivity was achieved by tandem mass spectrometric analysis.

Raja Kumar Seshadri *et al.*[104] **(2010)**, reported **"Simultaneous Quantitative Determination of Metoprolol, Atorvastatin and Ramipril in Capsules by a Validated Stability- Indicating RP-UPLC Method"**. The developed Reverse Phase Ultra Performance Liquid Chromatographic method used Zorbax XDB-C_{18} column (4.6 mm X 50 mm, 1.8 µm) with mobile phase consisting of 0.06% ortho phosphoric acid in Milli Q water having an ion pair reagent, 0.0045 M sodium lauryl sulphate as buffer, at the ratio of buffer: Acetonitrile (50:50), at a flow rate of 1 ml/min and monitored at 210 nm.

Prasada Rao CH.M.M *et al.*[105] **(2010),** reported **"RP-HPLC Method of Simultaneous Estimation of Amlodipine Besylate and Metoprolol in Combined Dosage Form".** An

Inertsil ODS-CV column was used for chromatographic separation, with mobile phase consisting of 0.02 M phosphate buffer solution and Acetonitrile in the ratio of 80: 20, at a flow rate of 1 ml/min. The photo diode array detection was at 215 nm.

Suresh Kumar K *et al.*[106] **(2010),** reported "**Simultaneous Spectrophotometric Determination of Metoprolol Tartrate and Ramipril".** The double-beam Shimadzu UV- Visible spectrophotometer (Model 1700 Pharmaspec) was used. The absorbance maxima of Metoprolol Tartrate and Ramipril showed maximum absorption at 209.5 nm and 222 nm, respectively.

Yilmaz B[107] **(2010)** reported **"Determination of Metoprolol in Pharmaceutical Preparations by Zero-, First-, Second- And Third-Order Derivative Spectrophotometric Method".** The four methods show the high reliability and reproducibility. The best outcome were obtained at 276 nm, 285 nm, 282 nm and 281 nm for zero-, first-, second- and third-order derivative spectrophotometric methods.

Chandra Bose RJ *et al.*[108] **(2011),** reported **"Validated RP-HPLC Method for the Simultaneous Estimation of Ramipril and Metoprolol Tartrate in Bulk and Tablet Dosage Form".** The chromatographic separation was made on Hypersil C_{18} (150 mm X 4.6 mm, 5 μm) in isocratic mode with mobile phase consisting of acetonitrile: methanol: 10mM acetate buffer (30: 50: 20), pH adjusted to 5 ± 0.1 with triethanolamine, at a flow rate of 1.0 ml/min and absorbance was measured at 210 nm.

Bhargavi Durga K *et al.*[109] **(2011),** reported "**RP- HPLC Method for Estimation of Metoprolol in Bulk Drug"**. The chromatographic separation was carried out on C_{18} column (250 mm X 4.6 mm), with the mobile phase comprising of acetonitrile: water in the ratio of 50:50, at the flow rate of 1.0 ml/min and monitored at 220 nm.

Boyka G. Tsvetkova *et al.*[110] **(2012),** reported **"Development and Validation of RP-HPLC Method for Simultaneous Determination of Metoprolol and Aspirin in Fixed Dose Combinations"**. Chromatographic separation was performed isocratically with a LiChrosorb C_{18} (250 mm X 4.6 mm, 5 μm) column eluted with a mixture of phosphate buffer, pH 4.6 and methanol (20:80) as the mobile phase, at a flow rate of 0.8 ml/min. Detection was carried out by absorbance at 230 nm.

Jadhav AS *et al.*[111] **(2012),** reported **"Quantitative Determination of Metoprolol Succinate in Bulk and Tablet Dosage form through Comparative Study of UV and Derivative Spectroscopy".** The developed UV spectrophotometric methods showed maximum absorption at 223 nm and 226 nm for method I and method II respectively.

Nawale PS *et al.*[112] **(2012),** reported **"Normal and Reversed-Phase HPTLC Methods for Simultaneous Estimation of Telmisartan and Metoprolol Succinate in Pharmaceutical Formulation".** The methods employed are 1. Normal-phase and 2. Reversed-phase HPTLC/densitometry method for simultaneous determination of Telmisartan and Metoprolol Succinate in bulk and in combined tablet formulation. Method I was performed on aluminium plates precoated with silica gel 60 F254S, with mobile phase consisting of toluene: propanol: methanol: triethylamine (8: 1: 1: 0.5). In Method II, aluminium coated with RP-18 silica gel 60 F254S HPTLC plates and methanol: water: triethylamine (6: 4: 0.5) as mobile phase was used. Densitometric scanning was performed at 242 nm for both methods.

Bhangale YS *et al.*[113] **(2012),** reported **"A Validated HPTLC Method for Simultaneous Estimation of Metoprolol Succinate and Isosorbide Mononitrate in Combined Capsule Dosage Form".** The developed and validated method was carried

out using Methanol: Ethyl acetate: Triethylamine (6: 4: 0.1) as mobile phase on precoated Silica Gel 60 F254. The densitometric evaluation of bands was monitored at 215 nm.

Chitlange SS *et al.*[114] **(2012),** reported "**Development and Validation of Spectrophotometric and Stability Indicating RP-HPLC Method for the Simultaneous Estimation of Metoprolol Succinate and Hydrochlorothiazide in Tablet Dosage Form".** The developed methods are 1. UV-Spectrophotometric and 2. Reverse Phase-High Performance Liquid Chromatography. The UV methods employed are Absorption corrected for interference method and Multi-Component mode method at 276 nm and 316.5 nm for Metoprolol Succinate and Hydrochlorothiazide respectively. The RP-HPLC analysis was carried out on Thermo C_{18} column (4.6 mm X 250 mm), using 0.05M potassium dihydrogen orthophosphate buffer, pH 3 and acetonitrile in the ratio of 80: 20 as the mobile phase, at a flow rate of 1.1 ml/min, and detected at 222 nm.

3.2.6. REPORTED METHODS OF OLMESARTAN MEDOXIMIL:

Liu D *et al.*[115] **(2007),** reported **"Quantitative Determination of Olmesartan in Human Plasma and Urine by Liquid Chromatography Coupled to Tandem Mass Spectrometry".** Isolation of compounds from biological matrix was carried out by Solid-phase extraction. Chromatographic separation of injected extracts was carried out on Thermo BDS Hypersil C18 column (50 mm X 4.6 mm, 3µm) protected by Thermo BDS Hypersil C18 guard column (4 mm X 4.6 mm, 3µm), with mobile phase consisting of formic acid/methanol/water (0.5/70/30), at a flow rate of 0.6 ml/min. Mass chromatograms were recorded using an API 3000 triple-quadrupole mass spectrometer equipped with Turbo Ion Spray interface.

Murakami T *et al.*[116] **(2008),** reported **"Identification of a Degradation Product in Stressed Tablets of Olmesartan Medoxomil by the Complementary Use of HPLC Hyphenated Techniques".** The structure of degradation product (DP-1) in stressed Olmesartan Medoxomil tablets was elucidated by the hyphenated techniques of LC-MS, solvent-elimination LC-IR and LC-NMR using LC conditions compatible with each technique. The molecular formula and substructural information of the degradation product were identified by LC-MS and MS/MS spectra, and the presence of the ester functional group was determined by LC-IR analysis. The structure of the degradation product was confirmed by LC-NMR.

Najma S *et al.*[117] **(2008),** reported **"Simultaneous Determination of Olmesartan Medoxomil and Irbesartan and Hydrochlorothiazide in Pharmaceutical Formulations and Human Serum using High Performance Liquid Chromatography".** The validated HPLC method of analysing Olmesartan Medoxomil, Irbesartan and Hydrochlorothiazide in human serum and tablet formulation used µ Bondapak, C_{18} (15 cm X 4.6 mm, 5 µm), with mobile phase consisted of acetonitrile: 0.2% acetic acid aqueous solution (50: 50) under isocratic conditions at a flow rate of 1.0 ml/min, and detected at 260 nm.

Syed Shanaz Qutab *et al.*[118] **(2009),** reported **"Simultaneous Quantitation of Olmesartan Medoxomil and Amlodipine Besylate in Combined Tablets using HPLC".** High Performance Liquid Chromatography method was performed using reverse phase isocratic elution with C_{18} Column with a mobile phase consisting of 0.05 M ammonium acetate, pH 6.8 and acetonitrile in the ratio of 40: 60 at 239 nm.

Mehulkumar P *et al.*[119] **(2009),** reported **"Simultaneous Spectroscopic Estimation of Amlodipine Besylate and Olmesartan Medoximil in Tablet Dosage Form."** The developed first derivative zero crossing method for simultaneous determination showed zero crossing point at 237 nm and 259 nm for Amlodipine and Olmesartan respectively. This method is rapid, simple and specific.

Bari PD *et al.*[120] **(2009),** reported **"RP-LC and HPTLC Methods for the Determination of Olmesartan Medoximil and Hydrochlorothiazide in Combined Tablet Dosage Form".** In Reverse Phase Liquid Chromatography, the chromatographic separation was made on C_{18} column (250 X 4.6 mm, 5 µm) and the mobile phase consisting of methanol and 0.05% orthophosphoric acid (60:40), at a flow rate of 1.0 ml/min and detected at 270 nm. The second method involved silica gel 60 F_{254} high performance thin layer chromatography and densitometric detection at 254 nm with mobile phase as acetonitrile: ethyl acetate: glacial acetic acid (7: 3: 0.4)

Wankhede SB *et al.*[121] **(2009),** reported **"Simultaneous Estimation of Amlodipine Besilate and Olmesartan Medoximil in Pharmaceutical Dosage Form".** The developed methods were 1. UV Spectrophotometric Method and 2. Reverse Phase High Performance Liquid Chromatography. UV Spectrophotometric Method was based on Simultaneous equation method used 237.5 nm and 255.5 nm for Amlodipine Besilate (AMLO) and Olmesartan Medoximil (OLME) respectively. In the area under the curve method, 242.5-232.5 nm (for AMLO) and 260.5-250.5 nm (for OLME) were selected for analysis. RP-HPLC method was developed using Kromasil C_{18} Column (4.6 mm X 250 mm) with mobile phase consisting of 0.05 M potassium dihydrogen phosphate buffer: acetonitrile (50:50), at a flow rate of 1.0 ml/min and monitored at 238 nm.

Prabhat Jain *et al.*[122] **(2010),** reported **"Development and Validation of Spectrophotometric and RP-HPLC Method for Estimation of Olmesartan Medoximil in Tablet Dosage Form".** The methods employed are 1. Spectrophotometry and 2. Reverse Phase High Performance Liquid Chromatography. In Spectrophotometric method, Acetonitrile: Water (80: 20) was used as mobile phase and 258 nm was selected as sampling wavelength. HPLC method was developed using Lichrocart C_{18} Column (250 X 4 mm, 5µm) with methanol: acetonitrile, pH 4 as mobile phase at a flow rate of 1.0 ml/min. The eluent was monitored at 257 nm.

Desai DJ *et al.*[123] **(2010),** reported **"Validated HPTLC Method for Simultaneous Quantitation of Olmesartan Medoximil and Amlodipine Besylate in Bulk Drug and Formulation".** HPTLC analysis was performed on precoated silica gel aluminium plate 60 F_{254}, 254 µm with the solvent system of chloroform: methanol: toluene: acetic Acid (8: 1: 1: 0.1). Densitometric scanning was performed at 254 nm.

Chabukswar AR *et al.*[124] **(2010),** reported **"Development and Validation of RP-HPLC Method for Simultaneous Estimation of Olmesartan Medoximil and Amlodipine Besylate in Tablet Dosage Form".** Waters Symmetry C_{18} Column (250 mm X 4.6 mm, 5.0 µm) was used for chromatographic separation with mobile phase containing Acetonitrile: Methanol: Water (60: 28: 12), pH 3.2, at a flow rate of 0.6 ml/min, and detected at 254 nm.

Godse VP *et al.*[125] **(2010)** reported **"ICH guidance in practice: Validated Stability-Indicating HPLC Method for Simultaneous determination of Olmesartan Medoximil and Hydrochlorothiazide in combination drug products".** Degradation of both drugs together under different stress test conditions and the generated samples were

used to develop stability-indicating HPLC method. Separation of drugs from degradation products using Reverse Phase C_{18} column with mobile phase consisting of acetonitrile: phosphate buffer (pH 3.0), delivered initially 15:85 for 6 min, then changed to 30:70 for next 20 min, and finally equilibrated back to 15:85 from 20 to 25 min, at a flow rate of 1 ml/min. Detection was done at 258 nm and 224 nm for Olmesartan and Hydrochlorothiazide respectively.

Patil P *et al.*[126] **(2011),** reported **"Spectrophotometric Method for Simultaneous Determination of Olmesartan Medoximil and Amlodipine Besylate from Tablet Dosage Form".** The developed absorption correction method to estimate both the drugs in combination without previous separation was based on determination of Olmesartan at 265 nm and Amlodipine at 360 nm in acetonitrile and water.

Kardile DP *et al.*[7] **(2011),** reported **"Simultaneous Estimation of Amlodipine Besylate and Olmesartan Medoximil Drug Formulations by HPLC and UV-Spectrophotometric Methods**". The methods employed are 1. UV Derivative Spectrophotometric determination and 2. Reverse Phase High Performance Liquid Chromatography. The developed UV spectrophotometric method used simultaneous equation method at 239 nm and 256 nm for Amlodipine Besylate and Olmesartan Medoximil respectively. In RP-HPLC method, the chromatographic separation was made on C_{18} bonded phase (4.6 mm X 250 mm, 5 µm), with mobile phase comprising of 0.05 M potassium dihydrogen phosphate: Acetonitrile (50:50), pH 6.8 and monitored at 230 to 260 nm.

Kumanan R *et al.*[127] **(2011),** reported **"Stability Indicating RP-HPLC Method Development and Validation of Olmesartan Medoximil"**. The developed and validated

stability indicating Reverse Phase High Performance Liquid Chromatography used Luna C_{18} column with mobile phase as acetonitrile: 0.05 M potassium dihydrogen phosphate (50:50), pH 4.5 at a flow rate of 1.0 ml/min, and detected at 258 nm.

Baldania SL *et al.*[128] **(2012)**, reported **"Simultaneous Estimation of Metoprolol Succinate and Olmesartan Medoxomil in Pharmaceutical Formulation by Thin-Layer Chromatographic-Densitometric Method".** The Chromatographic separations were carried out on prepared aluminium HPTLC plates precoated with silica gel G 60 F254 and the plates were developed with methanol-ethyl acetate -toluene-glacial acetic acid in the ratio of 2.5: 3: 4.5: 0.3 as mobile phase. The developed chromatograms were detected and evaluated densitometrically at 224 nm.

3.2.7. REPORTED METHODS OF ASPIRIN:

Shah DA *et al.*[129] **(2007),** reported **"Development and Validation of a RP-HPLC Method for Determination of Atorvastatin Calcium and Aspirin in a Capsule Dosage Form".** A Phenomenex Gemini C_{18} column (250 X 4.6 mm, 5 µm) was used for chromatographic separation with mobile phase comprising of 0.02 M potassium dihydrogen phosphate-methanol (20: 80), pH 4 at a flow rate of 1 ml/min and the effluents was monitored at 240 nm.

Patole SM *et al.*[130] **(2007)** reported **"A Validated HPLC Method for Analysis of Atorvastatin Calcium, Ramipril and Aspirin as the Bulk Drug and in Combined Capsule Dosage Form".** Chromatographic separation was performed on JASCO chromatographic system with C_{18} column (250 mm X 4.6 mm) and mobile phase consisting of methanol: acetate buffer (70: 30), pH 3.1 at a flow rate of 1 ml/min.

Detection wavelength used was 210 nm for Ramipril, 245 nm for Atorvastatin and 254 nm for Aspirin.

Jose Luiz Neves de Aguiar *et al.*[131] **(2009)** reported **"Development of a new analytical method for Determination of Acetylsalicylic and Salicylic Acids in Tablet by Reversed Phase Liquid Chromatography"**. The developed method was used to determine the tenors of acetyl salicylic as well as salicylic acid in tablets. HPLC separation was achieved with C_{18} (150 X 3.9 mm, 4 µm) Novapack as stationary phase and acetonitrile: aqueous solution of trifluoroacetic acid, 0.05% (30:70) as eluent, at a flow rate of 1.0 ml/min, and monitored at 230 nm.

Gujarathi SC *et al.*[132] **(2010)** reported **"Spectrophotometric Simultaneous Determination of Aspirin and Ticlopidine in Combined Dosage Form by First Order Derivative Spectroscopy, Area under Curve (AUC) and Ratio Derivative Spectrophotometric Methods"**. The amplitudes at 232.98 nm and 239.5 nm in the first order derivative spectra were selected to determine Aspirin and Ticlopidine respectively. The wavelength ranges 234.15-238.88 nm and 215.30-219.50 nm were selected to determine Aspirin and Ticlopidine by AUC method. Amplitude at 224.61nm and 234.5 nm were selected in the ratio derivative spectra to determine Aspirin and Ticlopidine respectively.

Suresh Kumar S *et al.*[133] **(2010),** reported **"Analytical Method Development and Validation for Aspirin".** The developed and validated method for determination of Aspirin in the presence of its degradation product employed Hypersil BDS C_{18} column (100 X 4.6 mm, 5 µm) and sodium perchlorate buffer: acetonitrile: isopropyl alcohol

(85: 14: 1) as mobile phase, at a flow rate of 1.5 ml/min. UV detection was performed at 275 nm.

Pankaj Kumar *et al.*[134] **(2011)** reported **"Development and Validation of a Novel Isocratic RP-HPLC Method for Simultaneous Determination of Atenolol and Aspirin in Fixed Dose Combinations".** High Performance Liquid Chromatography system Adept series CECIL CE 4210 was employed with C_{18} column (250 mm X 4.6 mm) and Phosphate buffer: Methanol (85: 15), pH 4.5 as eluent, at a flow rate of 0.8 ml/min and detected at 239.5 nm.

Prakash K *et al.*[135] **(2011),** reported **"Rapid and Simultaneous Determination of Aspirin and Dipyridamole in Pharmaceutical Formulations by Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) Method"**. HPLC analysis was carried out on Waters symmetry C_{18} column (50 mm X 4.6 mm, 3.5 µm) in isocratic mode with mobile phase consisting of 0.1% orthophosphoric acid: acetonitrile (75: 25), at a flow rate of 1.0 ml/min and detected at 227 nm.

Murtaza G *et al.*[136] **(2011)** reported "**Development of a UV-Spectrophotometric Method for the Simultaneous Determination of Aspirin and Paracetamol in Tablets".** UV-Visible Spectrophotometer (1601, Shimadzu, Japan) was used. The method employed solving of simultaneous equations based on the measurement of absorbance at 265 nm and 257 nm for Aspirin and Paracetamol respectively.

Najma Sultana *et al.*[137] (**2011**), reported "**Simultaneous Determination of Clopidogrel and Aspirin by RP-HPLC from Bulk Material and Dosage Formulations Using Multivariate Calibration Technique**". The chromatographic analysis was performed on Purospher star C_{18} column (250 mm X 4.6 mm, 5 µm) and mobile phase consisting of

methanol-water (80: 20), pH 3.4 at a flow rate of 1 ml/min using isocratic pump system. The eluents were monitored at 225, 230, 235, 240, and 245 nm. Multivariate chromatographic calibration technique was subjected to HPLC data for simultaneous quantitative analysis of binary mixtures of Clopidogrel and Aspirin.

Krishnaiah V and Rami Reddy YV[138] (**2012**), reported "**Development and Validation of HPLC Method for the Simultaneous Determination of Aspirin**". The chromatographic separation of Aspirin, related substances and its degraded products was performed on BDS Hypersil C_{18} column (100 mm X 4. 6mm, 5 μm) with mobile phase comprising of sodium chlorate buffer (pH 2.5), acetonitrile and isopropyl alcohol (85:1:14) at a flow rate of 1.5 ml/min. The detection wavelength was 275 nm.

Ramakrishna Gajula *et al.*[139] **(2012)**, reported **"Simultaneous Determination of Atorvastatin and Aspirin in Human Plasma by LC-MS/MS: Its Pharmacokinetic Application"**. The developed and validated liquid chromatography tandem mass spectrometric assay method utilized liquid-liquid extraction technique for extraction of analytes from human plasma and the reconstituted samples were chromatographed on a Zorbax XDB Phenyl column with mobile phase consisting of 0.2% acetic acid buffer, methanol and acetonitrile (20: 16: 64) at a flow rate of 0.8 ml/min. Prior to detection, Atorvastatin and Aspirin were ionized using an ESI source in the multiple reaction monitoring (MRM) mode.

3.2.8. REPORTED METHODS OF ROSUVASTATIN:

Sonu Sundd Singh *et al.*[140] **(2005),** reported "**Estimation of Rosuvastatin in Human Plasma by HPLC Tandem Mass Spectroscopic Method and its Application to Bioequivalence Study**". Chromatographic estimation was performed on YMC J Sphere

ODS H-80 column (5150 mm X 4.6 mm, 4µm), with mobile phase consisting of acetonitrile: 0.2% formic acid in water (60: 40) at a flow rate of 1.0 ml/min with split of 200 µL to mass spectrophotometer and 800 µL to waste. The API 3000-LC-MS/MS system was operated in positive ion mode with Turbo ion spray heater set at 250°C.

Marothu Vamsi Krishna and Dannana Gowri Sankar[141] **(2007),** reported "**Extractive Spectrophotometric Methods for the Determination of Rosuvastatin Calcium in Pure Form and in Pharmaceutical Formulations by Using Safranin O and Methylene Blue".** Methods developed are based on the formation of ion association complexes of the Rosuvastatin with basic dyes Safranin O and Methylene blue.

Chaudhari BG *et al.*[142] **(2007),** reported "**Determination of Simvastatin, Pravastatin Sodium and Rosuvastatin Calcium in Tablet Dosage Forms by HPTLC".** The developed HPTLC method used precoated Silica gel $60F_{254}$ aluminium sheets as stationary phase and chloroform: methanol: toluene (6:2:2) as mobile phase. The wavelength scanning was performed at 239 nm, 238 nm and 310 nm for Simvastatin, Pravastatin sodium and Rosuvastatin respectively.

Hasumathi A.Raj *et al.*[143] **(2009),** reported **"Development and Validation of Two Chromatographic Stability-Indicating Methods for Determination of Rosuvastatin in Pure Form and Pharmaceutical Preparation".** Two Stability-indication methods were developed 1. High-Performance Liquid Chromatography and 2. High-Performance Thin Layer Chromatography. HPLC analysis was performed on Phenomenex C_{18} column (250 mm X 4.6 mm, 5 µm) with mobile phase consisting of acetonitrile: 0.5% formic acid (50: 50) at a flow rate of 1.0 ml/min, and monitored at 248 nm. HPTLC analysis was carried on silica gel 60 F_{254} plates in which the drug is separated from its acid degradation

products using ethyl acetate: toluene: acetonitrile: formic acid (6: 3.5: 0.5:0.2) as mobile phase, with UV detection at 243 nm.

Alka Gupta *et al.*[144] **(2009),** reported **"Simple UV Spectrophotometric Determination of Rosuvastatin Calcium in Pure form and in Pharmaceutical Formulations".** A GBC Cintra-10 double beam UV-Visible Spectrophotometer was used with the solvent methanol. The maximum absorption of Rosuvastatin was exhibited at 244 nm.

Doshi N *et al.*[145] **(2010),** reported **"Validated RP-HPLC Method for Simultaneous Estimation of Rosuvastatin Calcium and Telmisartan in Pharmaceutical Dosage Form".** HPLC separation was carried out on Inertsil ODS 3V C_{18} column (250 X 4.6 mm, 5 µm) with mobile phase comprising of ammonium dihydrogen phosphate (pH 3) buffer solution: Methanol (65: 35, pH 3) at a flow rate of 1.5 ml/min and monitored at 298 nm.

Gajjar Anuradha K and Shah Vishal D[146] **(2010),** reported **"Simultaneous UV-Spectrophotometric Estimation of Rosuvastatin and Ezetimibe in their Combined Dosage Forms".** The methods employed for simultaneous determination of Rosuvastatin an Ezetimibe were 1. Q-absorption Ratio Method, 2. Dual Wavelength method and 3. First Derivative Spectroscopy Method. A double-beam Shimadzu UV-Visible Spectrophotometer, Model UV-2450 PC was used with Methanol as solvent.

Pandya CB *et al.*[147] **(2010)**, reported **"Development and Validation of RP-HPLC Method for Determination of Rosuvastatin Calcium in Bulk and Pharmaceutical Dosage Form".** The chromatographic estimation was performed on Thermo hypersil reversed phase C_{18} column (100 X 4.6 mm, 5 µm) in gradient mode with mobile phase

comprising Acetonitrile: Potassium dihydrogen orthophosphate (50:50), pH 3 at a flow rate of 0.5 ml/min and effluents were monitored at 243 nm.

Patel B *et al.*[148] **(2010),** reported **"Comparative In-vitro Dissolution Study of Rosuvastatin Calcium and Telmisartan".** The in-vitro dissolution rates of 10 and 40 mg Rosatel tablets as sample from in house production of company and sample of innovator were measured in various dissolution medias using the rotating paddle apparatus. The similarity factor and dissimilarity factor for both drugs was found out and release profile media as well chromatographic conditions were found out.

Sultana N *et al.*[149] **(2010),** reported **"Simultaneous Determination of Ceftriazone Sodium and Statin Drugs in Pharmaceutical Formulations and Human Serum by RP-HPLC".** The developed Reverse Phase Liquid Chromatography for the simultaneous determination of Ceftriaxone, Simvastatin, Rosuvastatin, Atorvastatin and Pravastatin bulk drug materials, dosage formulations and in human serum utilized Purospher star C_{18} column (25 cm X 0.46 cm, 5 µm) with mobile phase comprising of methanol: water: acetonitrile (70: 15: 20), pH 2.8 at a flow rate of 1.0 ml/min. UV detection was performed at 240 nm.

Suresh Kumar GV *et al.*[150] (**2010**) reported "**Development and Validation of Reversed-Phase HPLC Method for Simultaneous Estimation of Rosuvastatin and Fenofibrate in Tablet Dosage Form**". HPLC separation was performed on symmetry C_{18} column (250 X 4.6 mm, 5 µm) with water: acetonitrile in the ratio of 30:70 as mobile phase at 1.0 ml/min. The dual wavelength was set, 248 nm and 286 nm for Rosuvastatin and Fenofibrate respectively.

Arm M. Badawy *et al.*[151] **(2011)** reported **"Stability Indicating Spectrophotometric Methods for Determination of Rosuvastatin in the Presence of its Degradation Products by Derivative Spectrophotometric Techniques".** Shimadzu UV-1601 PC, dual beam UV-Visible Spectrophotometer was used. Rosuvastatin was degraded by methanol and hydrochloric acid. Two methods 1. First derivative method and 2. Derivative ratio spectrophotometric method were employed to determine Rosuvastatin in the presence of its degradation products.

Devika GS *et al.*[152] **(2011),** reported **"A New Improved RP-HPLC Method for Simultaneous Estimation of Rosuvastatin Calcium and Fenofibrate in Tablets".** Phenomena C_{18} column (250 mm X 4.6 mm, 5 µm) was used for chromatographic separation with mobile phase consisting of Methanol: 0.02M ammonium dihydrogen phosphate buffer (75: 25), pH 5.5 at a flow rate of 1 ml/min and monitored at 272 nm.

Uma Devi S *et al.*[153] **(2011)**, reported **"Development and Validation of HPTLC Method for Estimation of Rosuvastatin Calcium in Bulk and Pharmaceutical Dosage Forms"**. HPTLC analysis was performed on silica gel 60 F_{254} plates with mobile phase consisting of ethyl acetate: toluene: methanol in the ratio of 6:2:2. Densitometric scanning was performed at 243 nm.

Dipali Tajane *et al.*[154] (**2012**), reported "**Development and Validation of a RP-HPLC-PDA Method for Simultaneous Determination of Rosuvastatin Calcium and Amlodipine Besylate in Pharmaceutical Dosage Form**". The developed and validated RP-HPLC-PDA method utilized Kromasil C_{18} column (250 mm X 4.6 mm, 5.0 µm) with mobile phase consisting of acetonitrile: THF: water, pH 3 (68: 12: 20) at a flow rate of

0.5 ml/min in isocratic mode and monitored at 251 nm. The peak purity was checked with the PDA.

Harshal Kanubhai Trivedi and Mukesh C. Patel[155] (**2012**), reported "**Development and Validation of a Stability-Indicating RP-UPLC Method for Determination of Rosuvastatin and Related Substances in Pharmaceutical Dosage Form**". The chromatographic separation was achieved on an Acquity BEH C18 (100 mm X 2.1 mm, 1.7 µm) column with mobile phase containing a gradient mixture of solvent-A (0.1% trifluoroacetic acid) and solvent-B (methanol). The eluents were monitored at 240 nm. The degradation of Rosuvastatin was studied under various stress conditions. Four major unknown degradation products (late eluting impurities) were found in acid stress condition and two unknown degradation products were found in oxidative stress condition.

Nadia M. Mostafa *et al.*[156] (**2012**), reported "**Stability-Indicating Methods for the Determination of Rosuvastatin Calcium in the Presence of its Oxidative Degradation Products**". Four different methods were developed. The first method is second derivative method at 243.6 nm. The second method is based on ratiospectra 1st derivative spectrophotometry of the drug at 240 nm. The third method was based on quantitative densitometric evaluation of thin-layer chromatography with mobile phase consisting of ethyl acetate: methanol: ammonia (7: 3: 0.01) and scanned at 245 nm. The fourth method is an HPLC method with mobile phase consisting of water: acetonitrile: methanol (40:40:20) using UV detection at 245nm.

Najma Sultana *et al.*[157] (**2012**), reported "**An Ultra-Sensitive LC Method for Simultaneous Determination of Rosuvastatin, Alprazolam and Diclofenac Sodium in**

API, Pharmaceutical Formulation and Human Serum by Programming the Detector". The chromatographic separation was achieved on Bondapak C_{18} column (25 cm X 0.46 cm, 10 μm) with mobile phase consisting of methanol: water (80: 20), pH 2.9 at a flow rate of 1 ml/min. The detection response was monitored at 240 nm.

Rekha Rajeevkumar *et al.*[158] (**2012**), reported "**Novel Simultaneous Determination of Rosuvastatin Calcium and Fenofibrate in Tablet Formulation by Derivative Spectrophotometry**". Shimadzu UV-1700 UV-visible spectrophotometer was used. UV spectrum of Rosuvastatin and Fenofibrate were derivatised to first order with $\Delta\lambda$=1 for the entire spectrum. Zero crossing points for Rosuvastatin and Fenofibrate was found to be 233.5 nm and 254 nm respectively.

Scope

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Plan of work

4. SCOPE AND PLAN OF WORK

The overall scope and plan of the research work is to develop the methods for new drug combinations enter into the markert and to validate the newer analytical methods as per ICH guidelines. The parameters used to validate the developed method are: Accuracy, Precision, Linearity, Range, Repeatability, Reproducibility, Limit of Detection, Limit of Quantitation and Ruggedness. The system suitability test parameters like Capacity factor, Asymmetry factor, Tailing factor, Theoretical plates, HETP and Resolution should be calculated for RP-HPLC chromatograms and compared with standard values.

The plan of the present work is listed below:

For UV method:

1. Find the solubility of drugs in various solvents
2. To determine maximum absorbance and selection of wavelengths for detection.
3. To determine the stability of drugs in the selected solvent at the specified wavelength
4. Determining the standard absorbance for all selected wavelengths for each drug
5. Development of simple, precise, accurate and sensitive methods
6. Validation of developed methods as per ICH guidelines.

For Reverse Phase High-Performance Liquid Chromotography method:

1. Selection of suitable mobile phase and common wavelength for two drugs with proper resolution and short duration of time
2. To determine the stability of the drugs in the mobile phase at the selected wavelength.
3. Relating the area of chromatogram with respect to concentration for individual drugs
4. Determination of percentage purity of physical mixture and in formulation
5. Validation of the developed method

Materials

&

Methods

5. MATERIALS AND METHODS

5.1 INSTRUMENTS SPECIFICATIONS

1. Shimadzu AUX- 200 digital balance
2. Shimadzu 1700 double beam UV-visible spectrophotometer with a pair of 10 mm matched quartz cells
3. Shimadzu HPLC system (LC-10ATVP)
4. Elico SL-210 double beam UV-visible spectrophotometer with a pair of matched quartz cells
5. Remi centrifuge apparatus
6. Sonicator model 2120 MH
7. Cyberlab micropipette
8. Elico LI 120 pH meter
9. Melting point apparatus - Guna enterprises Chennai

5.1.1 SPECIFICATIONS (TERMS) OF INSTRUMENTS

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

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

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

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

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

c) Shimadzu High Performance Liquid Chromatography:

(Shimadzu instruction manual)

Detector Specifications	
Light source	Deuterium Arc lamp
Measurement wavelength range	190 to 700 nm
Spectral Band Width	5 nm
Wavelength Accuracy	± 1 nm
Cell path length	10 nm
Cell volume	20 µl
Operating temperature range	4 to 35° C (39 to 104° F)
Recording range	0.0001 to 4.000 AUFS
Operating temperature/Humidity	4 to 35° C / 75 %
Pump Specifications	
Pump type	Double reciprocating plunger pump
Pumping method	Constant flow delivery and constant pressure delivery
Suction filter	45 µm
Line filter	5 µm mesh
Operating temperature	4 to 35° C (39 to 104° F)

5.2 REAGENTS AND CHEMICALS USED IN THE STUDY:

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

- Distilled water

- Acetonitrile (HPLC Grade)
- Methanol (Spectral and HPLC Grade)
- Water (Spectral and HPLC Grade)
- Orthophosphoric acid (Analytical Grade)

5.3 MATERIALS AND METHODS FOR DOXOFYLLINE AND AMBROXOL HYDROCHLORIDE COMBINATION DOSAGE FORM:

Drugs

Pharmaceutically pure sample of Doxofylline (DOX) and Ambroxol Hydrochloride (AMB) were generously gifted by Shine Pharmaceuticals Pvt. Ltd., Chennai and Apex Pharmaceuticals Pvt. Ltd., Allathur. Combination product (SYNASMA-AX, Ranbaxy Laboratories Ltd.) containing 400 mg Doxofylline and 30 mg Ambroxol Hydrochloride was procured from a local Pharmacy.

Methods Employed

The methods employed for the simultaneous estimation of Doxofylline and Ambroxol Hydrochloride in combination are

1. UV Spectrophotometric method

a. Simultaneous equation method

b. Absorbance correction method and

c. Absorbance Ratio Method

2. Reverse Phase High-Performance Liquid Chromotography method

5.3.1. UV SPECTROPHOTOMETRIC METHODS:

Selection of solvent

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

Preparation of standard stock solution

Accurately weighed drug samples of both DOX and AMB (50 mg each) were transferred to a suitable standard volumetric flask separately, dissolved and diluted to mark with distilled water. Both the drug solutions were diluted so as to get 10 µg/ml. The solutions were scanned in the UV region of 200-400 nm in 1cm cell against distilled water as blank and the overlain spectra was recorded.

Selection of wavelengths for estimation and stability studies

From the overlain spectra, by the observation of spectral characteristics of DOX and AMB, the drugs were simultaneously estimated by Simultaneous equation method, Absorbance correction method and Absorbance Ratio method. The wavelengths selected for Simultaneous equation method were 274 nm and 244.5 nm for DOX and AMB respectively.

For Absorbance Correction Method, it was observed that DOX has zero absorbance at 308 nm, where as AMB has substantial absorbance. Thus AMB was estimated directly at 308 nm without interference of DOX. For estimation of DOX, the absorbance of AMB was measured at 274 nm using standard solution of 10 µg/ml. The contribution of AMB

was deducted from the total absorbance of sample mixture at 274 nm. The calculated absorbance was called as corrected absorbance for DOX. To estimate the amount of DOX, the absorbance of AMB were corrected for interference at 274 nm by using absorptivity values.

For Absorbance Ratio Method, the wavelengths selected were 244.5 nm (λ_{max} of AMB) and 233.5 nm (wavelength of equal absorptivity of two components i.e. iso-absorptive point).

Preparation of calibration graph

From the above stock solution, aliquots were drawn and suitably diluted so as to get the final concentration range of 7-35 µg/ml of DOX and 1-5 µg/ml of AMB. Absorbances of these solutions were recorded in the respective wavelengths.

Quantification of tablet formulation

Twenty tablets were weighed and the average weight was found. The tablets were triturated to get a fine powder. An accurately weighed quantity of powder equivalent to 70 mg of DOX was transferred into a 100 ml volumetric flask, sufficient 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 and the filterate was suitably diluted to get final concentration of 14 µg/ml of DOX and 1 µg/ml of AMB with distilled water. The absorbance of sample solution was measured at all selected wavelengths. The content of DOX and AMB in sample solution of tablet was calculated. This procedure was repeated six times.

Recovery studies

The recovery experiment was done by adding known concentrations of DOX and AMB raw materials to the 50% preanalyzed formulation. Standard DOX and AMB in the range of 80%, 100% and 120% to the 50% preanalyzed formulation into a series of 10 ml volumetric flasks and diluted with distilled water and made up to the mark with the same. The contents were sonicated for 15 minutes. After sonication, the solutions were filtered through Whatmann filter paper No. 41. The absorbances of the resulting solutions were measured at their selected wavelengths for determination of DOX and AMB. The amount of each drug recovered from the formulation was calculated for all the drugs by Simultaneous equation method, Absorbance correction method and Absorbance ratio method. The procedure was repeated for three times for each percentage recovery.

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 7-35 µg/ml and AMB showed linearity in the range of 1-5 µg/ml and the calibration curves [mean value of six determinations] were plotted between concentration and absorbance of drugs. Optical parameters were calculated.

Accuracy (Recovery studies)

To check the accuracy of the developed method and to study the interference of formulation excipients, analytical recovery experiments were carried out by using standard addition method in three different concentrations. From the total amount of drug found, the percentage recovery and %RSD were calculated.

Precision:

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

Ruggedness:

The ruggedness test of analytical assay method is defined as 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, different lots of reagents etc. Ruggedness is a measure of reproducibility of test results under normal expected operational conditions from laboratory to laboratory and from Analyst to Analyst. In present study, determination of the DOX and AMB were carried out by using different instruments and different Analysts.

5.3.2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD:

Chromatographic method depends upon the nature of the sample, molecular weight and solubility. The drugs selected for the present study was polar compound; hence it can be separated either by normal phase or reverse phase chromatography. Reverse phase chromatographic technique was selected for initial separations with the knowledge of properties of compounds. C_{18} column was chosen as stationary phase and various mixtures of phosphate buffer (pH 3.0), acetonitrile and methanol were selected as mobile phase.

Selection of mobile phase and λ_{max}

Different mixtures of mobile phase with different ratios were selected and their chromatograms were recorded. From this, the mobile phase selected for the study was 10 mM Phosphate buffer, Acetonitrile and Methanol in the ratio of 70: 20: 10 and the pH is adjusted to 3.0 with orthophosphoric acid, since these two drugs were eluted with sharp peak and with better resolution. Hence, this mobile phase was used to optimize the chromatographic conditions. The detection wavelength was measured by scanning the 10 µg/ml solution of Doxofylline and Ambroxol HCl in the mobile phase in UV-Spectrophotometry, and overlaid spectra was recorded. The detection wavelength selected was 224 nm (isoabsorptive point of two drugs).

Optimized Chromatographic Conditions

The following parameters were used for RP-HPLC analysis of DOX and AMB

Mode of operation - Isocratic

Stationary phase - C_{18} column (150 mm X 4.6 mm I.d., 5m)

Mobile phase - 10 mM Phosphate buffer, Acetonitrile and Methanol (70: 20: 10)

Detection wavelength- 224 nm

Flow rate- 1 ml/min

Temperature- Ambient

Sample volume- 20 µl

Operating pressure- 138 kgf

Preparation of the Standard stock solution

Standard Doxofylline stock solution

Accurately weighed 35 mg of DOX was transferred into a 10 ml standard volumetric flask separately and dissolved with minimum quantity of HPLC water and the volume was made up to the mark with HPLC water. From the above solution, 1 ml was transferred into a 50 ml volumetric flask and diluted with HPLC water to get the concentration of 70 µg/ml of DOX.

Standard Ambroxol Hydrochloride solution

Accurately weighed 25 mg of AMB was transferred into a 10 ml standard volumetric flask separately and dissolved with minimum quantity of HPLC water and the volume was made upto the mark with HPLC water. From the above solution, 1 ml was transferred into a 10 ml volumetric flask and diluted with HPLC water to get the concentration of 250 µg/ml. From this solution, 1 ml was transferred into a 50 ml volumetric flask and diluted with HPLC water to get the final concentration of 5 µg/ml of AMB.

Linearity and Calibration

Aliquots (1-5 ml) of mixed working standard solutions of DOX and AMB were transferred into a series of 10 ml volumetric flasks, and the volume was made up to the mark with distilled water. An aliquot (20 μl) of each solution was injected under the operating chromatographic condition as described above and the responses were recorded. Calibration curves were constructed for each drug by plotting peak area versus concentration, and the regression equations were calculated. Each response was average of three determinations.

Quantification of tablet formulation

Twenty tablets containing DOX 400 mg and AMB 30 mg were accurately weighed. Weighed content of drug equivalent to 35 mg of DOX was transferred into a 10 ml volumetric flask and dissolved with HPLC water and sonicated for 15 minutes. The above solution was filtered through Whatmann filter paper No. 41 and the clear solution was collected. HPLC water is added to made up to the required volume to get the concentration of 3.5 mg/ml. 1 ml was pipetted into a 50 ml volumetric flask and made up to the mark with HPLC water to get the concentration of DOX (70 μg/ml) and AMB (5 μg/ml). Accurately measured 2 ml of the sample solution was transferred into a 10 ml volumetric flask, and diluted up to the mark with HPLC water to get the final working concentration of DOX (14 μg/ml) and AMB (1 μg/ml). The peak area measurements were done by injecting sample six times and the amount of DOX and AMB were calculated from their respective calibration curve.

Recovery Studies

The accuracy of the method was determined by calculating the recoveries of DOX and AMB by the standard addition method. Known amounts of standard solutions of DOX and AMB were added at 80%, 100% and 120% level to prequantified sample solution of DOX (14 μg/ml) and AMB (1 μg/ml). The amounts of DOX and AMB were estimated by applying obtained values to the respective regression equations.

Limit of Detection and Limit of Quantitation

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

System Suitability Studies

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

5.4 MATERIALS AND METHODS FOR METOLAZONE AND SPIRONOLACTONE COMBINATION DOSAGE FORM:

Drugs

Standard bulk drug samples of Metolazone (MET) and Spironolactone (SPIR) were provided by Centaur Pharmaceuticals, Mumbai. Combination product (METOLACTONE-5) containing 5 mg of Metolazone and 25 mg of Spironolactone were procured from a local Pharmacy.

Methods Employed

The methods employed for the simultaneous estimation of Metolazone and Spironolactone in combination were

1. UV Spectrophotometric method

a. Simultaneous equation method

b. Absorbance correction method and

c. First derivative spectroscopic method

2. Reverse Phase-High Performance Liquid Chromatography method

5.4.1 UV SPECTROPHOTOMETRIC METHODS

Selection of solvent

The solubility of drugs were determined in a variety of solvents as per Indian Pharmacopoeial standards. Solubility was carried out in non-polar to polar solvents. The common solvent was found to be methanol for the analysis of MET and SPIR for the developed method.

Preparation of standard stock solution

Accurately weighed drug samples of MET (50 mg) and SPIR (125 mg) were transferred to a 100 ml standard volumetric flask, dissolved and diluted to mark with methanol to get the concentration of 500 µg/ml for MET and 1250 µg/ml for SPIR. The solutions were further diluted with 0.02 M phosphate buffer, pH 3.5 adjusted with orthophosphoric acid to get 10 µg/ml and 50 µg/ml for MET and SPIR respectively.

Selection of wavelengths for estimation and stability studies

From the overlain spectra of MET (10 µg/ml) and SPIR (10 µg/ml) in 0.02 M phosphate buffer, pH 3.5 adjusted with orthophosphoric acid, wavelengths 236.5 nm (λmax of MET) and 242.5 nm (λmax of SPIR) were selected for the formation of simultaneous equation method.

In Absorbance Correction method, it was observed that SPIR have zero absorbance at 345 nm, where as MET has substantial absorbance. Thus MET was estimated directly at 345 nm without interference of SPIR. For estimation of SPIR, the absorbance of MET was measured at 242.5 nm using standard solution of MET (10 µg/ml). The contribution of MET was deducted from the total absorbance of sample mixture at 242.5 nm. The calculated absorbance was called as corrected absorbance for SPIR. To estimate the amount of SPIR, the absorbance of MET were corrected for interference at 242.5 nm by using absorptivity values.

In derivative spectroscopy determination, UV spectrum of both the drugs were derivatised to first order with $\Delta\lambda=1$ for the entire spectrum. This method involves first derivative spectroscopy using 266 nm and 289 nm as zero crossing points for MET and SPIR respectively.

Preparation of calibration graph of the drugs

The aliquots of stock solution of MET (0.5-2.5 ml of 10 µg/ml) and SPIR (1-5 ml of 50 µg/ml) were transferred into 10 ml volumetric flasks and made up to the volume with methanol. The absorbance of different concentration solutions were measured at 236.5 nm, 242.5 nm & 345 nm in the normal spectrum and 266 nm & 289 nm in the first

derivative spectrum for MET and SPIR. The calibration curve was plotted at their corresponding wavelengths. The drugs MET and SPIR were linear with the concentration range of 0.5-2.5 μg/ml and 5-25 μg/ml respectively at their respective wavelengths for Simultaneous equation method and Absorbance Correction method. For First order derivative method, Metolazone and Spironolactone were linear in the concentration range of 1-5 μg/ml and 10-50 μg/ml respectively.

Quantification of tablet formulation

Twenty tablets were weighed and average weight per tablet was determined. Tablets were grounded to a fine powder and accurately weighed tablet powder equivalent to 75 mg of MET was transferred into a volumetric flask. Sufficient methanol was added, sonicated for 15 min and diluted to the mark with 0.02 M phosphate buffer, pH 3.5 adjusted with orthophosphoric acid. It was filtered through Whatmann filter paper No. 41 and the filtrate was suitably diluted to get the required concentration of the linearity with 0.02 M phosphate buffer, pH 3.5 adjusted with orthophosphoric acid. Absorbances were measured at the said wavelengths, 236.5 nm and 242.5 nm in the simultaneous equation method, 266 nm and 289 nm in the first order spectrum for derivative method and at 242.5 nm and 345 nm for absorbance correction method and amount present was calculated using Simultaneous equation, First order derivative and Absorbance correction methods.

Recovery studies

The recovery experiment was done by adding known concentrations of MET and SPIR raw materials to the 50% preanalyzed formulation. Standard MET and SPIR in the range

of 80%, 100% and 120% to the 50% preanalyzed formulation into a series of 10 ml volumetric flasks and dissolved with methanol and made up to the mark with the same. The contents were sonicated for 15 minutes. After sonication, the solutions were filtered through Whatmann filter paper No. 41. The absorbances of the resulting solutions were measured at their selected wavelengths for the determination of MET and SPIR. The amount of each drug recovered from the formulation was calculated for all the drugs by Simultaneous equation method, Absorbance correction method and Derivative spectroscopic method. The procedure was repeated for three times for each percentage recovery.

Validation of developed method

The method was validated with respect to linearity, LOD (Limit of Detection), LOQ (Limit of Quantitation), Precision and Accuracy.

Linearity

Calibration curves were prepared for both the drugs at the selected analytical wavelengths. MET obeys Beer's law in the concentration range of 0.5-2.5 μg/ml and SPIR obeys Beer's law in the concentration range of 5-25 μg/ml for simultaneous equation method and absorbance correction method. Whereas MET obeys Beer's law in the concentration range of 1-5 μg/ml and SPIR obeys Beer's law in the concentration range of 10-50 μg/ml for First order Derivative spectroscopy.

Accuracy (Recovery studies)

To study accuracy, reproducibility and precision of the proposed methods, recovery studies were carried out by standard addition method. Results of recovery studies were

found to be satisfactory. Precision of the method was determined by performing Intraday (n=3) and Interday (n=3) analysis.

LOD and LOQ

LOD and LOQ were calculated in accordance with ICH guidelines, as 3.3 σ/S and 10 σ/S respectively, where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

Accuracy:

The accuracy of the method was determined by investigating the recovery of MET and SPIR, three levels ranging from 80%, 100% and 120% of the nominal concentration by standard addition technique.

Precision and Reproducibility:

The precision and repeatability of the method were studied by repeating the proposed method three times in a day, the average percentage and RSD values were determined. The results confirm the intraday and interday precision of the method. All the three methods are suitable for the reliable analysis of commercial formulations containing combinations of MET and SPIR. The methods are simple, precise, rapid and accurate. High percentage recovery shows that the method is free from the interference of excipients used in the formulation.

5.4.2. HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD:

Reverse-Phase High Performance Liquid Chromatographic technique was selected for initial separations with the knowledge of properties of compounds. C_{18} column was chosen as stationary phase and mixture of 25 mM phosphate buffer, acetonitrile and methanol (40: 30: 30) were chosen as mobile phase.

Selection of mobile phase and λ_{max}

Different mixtures of mobile phase with different ratios were selected and their chromatograms were recorded. From this, 25 mM phosphate buffer, acetonitrile and methanol (40:30:30) was selected as mobile phase, since these two drugs were eluted with sharp peak and with better resolution. Hence, this mobile phase was used to optimize the chromatographic conditions. The detection wavelength was measured by scanning the 10 μg/ml solution of MET and SPIR using mobile phase as solvent in the UV-spectrophotometry. An overlaid spectrum was made and the detection wavelength selected was 238 nm (common wavelength).

Optimized Chromatographic Conditions

The following parameters were used for RP-HPLC analysis of MET and SPIR

Mode of operation- Isocratic

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

Mobile phase- 25 mM phosphate buffer: Acetonitrile: Methanol (40:30:30)

Detection wavelength- 238 nm

Flow rate- 1 ml/min

Temperature- Ambient

Sample volume- 20 µl

Operating pressure- 164 kgf

Preparation of the Standard stock solution

Standard Metolazone stock solution

Weighed accurately 25 mg of MET was transferred into a 50 ml standard volumetric flask separately and dissolved with minimum quantity of methanol and the volume was made up to the mark with methanol. From the above solution, 1 ml was transferred into a 50 ml volumetric flask and diluted with methanol to get the concentration of 10 µg/ml for MET.

Standard Spironolactone stock solution

Weighed accurately 12.5 mg of SPIR and transferred into a 10 ml standard volumetric flask separately and dissolved with minimum quantity of methanol and the volume was made up to the mark with methanol. From the above solution, 1 ml was transferred to a 25 ml volumetric flask and diluted with methanol to get the concentration of 50 µg ml^{-1} of SPIR.

Linearity and Calibration

From the working standard solution, pipetted 0.5-2.5 ml of MET and 1-5 ml of SPIR into a series of five 10 ml volumetric flask and made up to the mark with mobile phase to obtain the concentration range from 0.5-2.5 µg/ml of MET and 5-25 µg/ml of SPIR. The solutions were injected and chromatograms were recorded. Calibration curves were

constructed by plotting the mean peak areas versus the concentration, and the regression equations were calculated. Each response was average of three determinations.

Quantification of tablet formulation

Twenty tablets containing MET and SPIR were accurately weighed. Weighed content of drug equivalent to 25 mg of SPIR was transferred into a 10 ml volumetric flask and dissolved in methanol and sonicated for 15 minutes. The final concentration was 2500 μg ml^{-1}. The above solution was filtered through Whatmann filter paper No. 41 and the clear solution was collected and 1 ml was pipetted out into a 25 ml volumetric flask and made up to the mark with methanol. From this, 1 ml was pipetted out into 10 ml volumetric flask and made up to mark with the mobile phase to produce 10 μg/ml solution. The peak area measurements were done by injecting sample six times and the amount of MET and SPIR were calculated from their respective calibration curve.

Recovery Studies

The accuracy of the method was determined by calculating the recoveries of MET and SPIR by the standard addition method. Known amount of standard solutions of MET and SPIR were added at 80, 100 and 120% level to prequantified sample solution of MET (1 μg/ml) and SPIR (10 μg/ml). The amount of MET and SPIR were estimated by applying obtained values to the respective regression equations.

Limit of Detection and Limit of Quantitation

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

System Suitability Studies

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

5.5 MATERIALS AND METHODS FOR METOPROLOL AND OLMESARTAN COMBINATION DOSAGE FORM:

Drugs

Pharmaceutically pure sample of Metoprolol (METO) and Olmesartan (OLME) were obtained from Caplinpoint Pvt. Ltd., Pondicherry. The combined dosage forms of Metoprolol and Olmesartan were procured from a local Pharmacy

BRAND NAME	DOSAGE FORM	COMPANY NAME	COMBINATION FORM
OLSAR-M 25	Capsule	Unichem Laboratories, Mumbai.	METO 25 mg + OLME 20 mg
OLMESAR-M	Tablet	Macleods Pharmaceuticals Ltd., Mumbai.	METO 25 mg + OLME 20 mg
OLMAX-M	Tablet	Glenmark Pharmaceuticals Ltd., (Healtheon), Mumbai.	METO 50 mg + OLME 20 mg

Methods Employed

1. UV Spectrophotometric methods

a. Simultaneous equation method

b. Area under the curve method and

c. First order derivative method

5.5.1 UV SPECTROPHOTOMETRIC METHODS:

Selection of solvent

The solubility of drugs were determined in a variety of solvents as per Indian Pharmacopoeial standards. Solubility was carried out in non polar to polar solvents. According to the solubility characteristics, the common solvents for the two drugs were found to be methanol.

Preparation of standard stock solution

Accurately weighed drug samples of both METO (25 mg) and OLME (20 mg) were transferred into a 10 ml volumetric flask separately, dissolved and diluted to mark with methanol to get the of 2.5 mg/ml of METO and 2 mg/ml of OLME respectively. From the above solutions, 1 ml was transferred into a 50 ml volumetric flask separately to get the final concentration of 50 µg/ml of METO and 40 µg/ml of OLME respectively.

10 µg/ml concentration of both drugs were scanned in the UV region of 200-400 nm in 1 cm cell against methanol as blank and the overlain spectra was recorded.

Selection of wavelengths for estimation and stability studies

From the overlain spectra of METO (10 µg/ml) and OLME (10 µg/ml) in methanol, the wavelengths 223.5 nm (λmax of METO) and 256.5 nm (λmax of OLME) were selected for the formation of Simultaneous Equation method.

For Area Under the Curve method, calibration curve was plotted after scanning in the UV region of 200-400 nm and the sampling wavelength ranges selected for estimation of METO and OLME are 218-228 nm (λ1-λ2) and 246-266 nm (λ3-λ4) and area were integrated between the selected wavelength ranges for both drugs which showed linear

response with increasing concentration. Hence the same wavelength ranges were used for estimation of capsule formulations.

In the first order derivative method, it was observed that METO showed dA/dλ zero at 243 nm in contrast to OLME that has considerable dA/dλ at this wavelength. Further OLME has dA/dλ zero at 256 nm, while at this wavelength METO has considerable dA/dλ. Therefore wavelengths 256 nm and 243 nm were employed for the determination of METO and OLME respectively.

Preparation of calibration graph

From the above stock solution, aliquots were drawn and suitably diluted so as to get the final concentration range of 5-25 µg/ml of METO and 4-20 µg/ml of OLME. Absorbances of these solutions were recorded in the respective wavelengths.

Quantification of the formulation

a. OLSAR-M 25 and OLMESAR-M:

Twenty capsules/tablets were weighed and average weight per capsule/tablet was determined. An accurately weighed quantity of powder in capsule/grounded tablet equivalent to 62.5 mg of METO in each brand was transferred into a 10 ml volumetric flask, sufficient methanol was added and the solution was sonicated for 15 minutes and diluted to the mark with methanol. It was filtered through Whatmann filter paper No. 41 and the filterate was suitably diluted to get final concentration of 10 µg/ml of METO and 8 µg/ml of OLME with methanol. The absorbance of sample solution was measured at all selected wavelengths.

b. OLMAX-M:

Twenty tablets were weighed and average weight per tablet was determined. An accurately weighed quantity of powder equivalent to 50 mg of METO was transferred into a 50 ml volumetric flask, sufficient methanol was added and the solution was sonicated for 15 minutes and diluted to the mark with methanol. It was filtered through Whatmann filter paper No. 41. From this filterate, 1 ml was transferred into 50 ml standard volumetric flask and diluted with methanol up to the mark to get the final concentration of 20 µg/ml of METO and 8 µg/ml of OLME. The absorbance of sample solution was measured at all selected wavelengths.

The contents of METO and OLME in sample solution of capsule powder/tablet were calculated, which was repeated six times.

Recovery studies

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

recovery. The same procedure was followed for all brands of combined dosage forms [OLSAR-M 25, OLMESAR-M and OLMAX-M].

Validation of developed method

The methods were validated with respect 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. METO was linear with the concentration range of 5-25 µg/ml and OLME showed linearity in the range of 4-20 µg/ml and calibration curve (mean value of six determinations) was plotted between concentration and absorbance of drugs. Optical parameters were calculated.

Accuracy (Recovery studies):

To check the accuracy of the developed method and to study the interference of formulation excipients, analytical recovery experiments were carried out by using standard addition method in three different concentrations. From the total amount of drug found, the percentage recovery was calculated. This procedure was repeated for three times for each concentration. The %RSD was also calculated. The accuracy of the developed method was carried out for all brands of combined dosage forms [OLSAR-M 25, OLMESAR-M and OLMAX-M].

Precision:

The precision of the method was confirmed by repeatability and intermediate precision. The repeatability was performed by the analysis of formulation and it was repeated for

six times with the same concentration. The amount of each drug present in the capsule/tablet formulation was calculated. The %RSD was calculated. The intermediate precision of the method was confirmed by intraday and interday analysis i.e. the analysis of formulation was repeated three times in the same day and on three successive days. The amount of drugs was determined and %RSD also calculated. It was carried out for all brands of combined dosage forms [OLSAR-M 25, OLMESAR-M and OLMAX-M].

Ruggedness:

The ruggedness test of analytical assay method is defined as 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, different lots of reagents etc. Ruggedness is a measure of reproducibility of test results under normal expected operational conditions from laboratory to laboratory and from Analyst to Analyst. In the present study, determination of METO and OLME were carried out by using different instruments and different Analysts and it was carried out for all brands of combined dosage forms [OLSAR-M 25, OLMESAR-M and OLMAX-M].

5.6 MATERIALS AND METHODS FOR ASPIRIN AND ROSUVASTATIN COMBINATION DOSAGE FORM:

Drugs

Pharmaceutically pure samples of Aspirin (ASP) and Rosuvastatin Calcium (ROSU) were generously gifted by Apex Pharmaceuticals Pvt. Ltd., Allathur, Chennai. Combination product (ROZUCOR ASP-10, Torrent Pharmaceuticals Pvt. Ltd.) containing 75 mg Aspirin and 10 mg Rosuvastatin and was procured from a local Pharmacy.

Methods Employed

The UV Spectrophotometric methods employed for the simultaneous estimation of Aspirin and Rosuvastatin in combination were

a. Simultaneous Equation Method and

b. Absorbance Ratio Method

5.6.1. UV SPECTROPHOTOMETRIC METHODS

Selection of solvent

The solubility of drugs were determined in a variety of solvents as per Indian Pharmacopoeial standards. Solubility was carried out in non polar to polar solvents. The common solvent was found to be methanol for the analysis of ASP and ROSU for the proposed method.

Preparation of standard stock solutions

Accurately weighed 75 mg of ASP was transferred into a 100 ml volumetric flask, dissolved and diluted to mark with methanol. From this, 5 ml was transferred into a 50 ml standard volumetric flask to obtain 75 µg/ml concentration solution.

Accurately weighed 25 mg of ROSU was transferred into a 50 ml standard volumetric flask and made up to the mark with methanol. From this, 1 ml was transferred into 50 ml standard volumetric flask to obtain 10 µg/ml concentration solution.

Selection of wavelengths for estimation and stability studies

The solutions of 10 µg/ml concentration of ASP and ROSU were scanned in the UV region of 200-400 nm individually and the overlaid spectrum was also recorded.

From the overlain spectrum of ASP and ROSU in methanol, wavelengths 294.5 nm (λmax of ASP) and 243 nm (λmax of ROSU) were selected for the Simultaneous Equation Method.

For Absorbance Ratio Method, the wavelengths selected were 243 nm (λ_{max} of ROSU) and 229.8 nm (iso-absorptive point of ASP and ROSU).

Preparation of calibration graph

From the above stock solution, aliquots were drawn and suitably diluted so as to get the final concentration range of 7.5-37.5 µg/ml of ASP and 1-5 µg/ml of ROSU. Absorbances of these solutions were recorded in the respective wavelengths for all methods.

Quantification of capsule formulation

Twenty capsules were weighed and the average weight of the powder was found. An accurately weighed quantity of the powder equivalent to 75 mg of ASP was transferred into a 100 ml volumetric flask, sufficient methanol was added and the solution was sonicated for 15 minutes and diluted to the mark with methanol. It was filtered through Whatmann filter paper No. 41 and the filterate was suitably diluted to get final concentration of 15 µg/ml of ASP and 2 µg/ml of ROSU with methanol. The absorbance of sample solution was measured at all selected wavelengths. The content of ASP and ROSU in sample solution of capsule was calculated. This procedure was repeated for six times.

Recovery studies

The recovery experiment was performed by adding known concentrations of ASP and ROSU raw materials to the 50% preanalyzed formulation. Standard ASP and ROSU in the range of 80%, 100% and 120% were transferred into the 50% preanalyzed formulation in a series of 10 ml volumetric flasks and diluted with methanol and made up to the mark with the same. The contents were sonicated for 15 minutes. After sonication, the solutions were filtered through Whatmann filter paper No. 41. The absorbances of the resulting solutions were measured at their selected wavelengths for the determination of ASP and ROSU. The amount of each drug recovered from the formulation was calculated for all the drugs by Simultaneous equation method and Absorbance ratio method. The procedure was repeated for three times for each percentage recovery.

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. ASP was linear with the concentration range of 7.5-37.5 µg/ml and ROSU showed linearity in the range of 1-5 µg/ml and the calibration curves [mean value of six determinations] were plotted between concentration and absorbance of drugs. Optical parameters were calculated.

Accuracy (Recovery studies)

To check the accuracy of the developed method and to study the interference of formulation excipients, analytical recovery experiments were carried out by using standard addition method in three different concentrations. From the total amount of drug found, the percentage recovery and %RSD were calculated.

Precision

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

Ruggedness

The ruggedness test of analytical assay method is defined as 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, different lots of reagents etc. Ruggedness is a measure of reproducibility of test results under normal expected operational conditions from laboratory to laboratory and from Analyst to Analyst. In the present study, determination of ASP and ROSU were carried out by using different instruments and different Analysts.

Results

&

Discussion

6. RESULTS AND DISCUSSION

In order to quench the thirst for the analysis of the new drug combinations, Doxofylline & Ambroxol Hydrochloride, Metolazone & Spironolactone, Metoprolol & Olmesartan Medoxomil and Aspirin & Rosuvastatin were taken for our studies. Simultaneous estimation of multiple drug formulations have advantage that the methods were less time consuming and the usage of solvent is minimized. To ensure the percentage purity in combined dosage forms of the drugs, the UV-spectroscopy, RP-HPLC or both were developed. These methods were found to be simple, economic and applicable for routine analysis.

DOXOFYLLINE AND AMBROXOL HYDROCHLORIDE COMBINATION DOSAGE FORM:

The methods employed for the analysis of Doxofylline and Ambroxol Hydrochloride were

1. UV-Spectroscopic Methods

a. Simultaneous equation method

b. Absorbance correction method and

c. Absorbance Ratio Method

2. Reverse-Phase High Performance Liquid Chromatography

6.1 UV-SPECTROSCOPIC METHODS:

The solubility of DOX and AMB were determined in a variety of solvents as per ScHefter and Higuchi method[159]. 10 mg of samples were taken in test tube and checked their solubility with variety of solvents as per IP and the profiles are shown in Table-1.

The numeral polar and non-polar solvents were attempted to dissolve the drugs. From the solubility profile, the distilled water was chosen as a common solvent for the estimation of DOX and AMB in bulk and in formulation.

Based upon its easy availability, cost factor and stability condition, distilled water was selected as solvent.

Three accurate, simple and rapid UV methods, namely Simultaneous equation method, Absorbance correction method and Absorbance ratio method were selected.

The drugs were dissolved in distilled water to produce 10 μg/ml. Scanned in the UV-region of 200-400 nm by using distilled water as blank, it shows constant wavelength at 274 nm for DOX and 244.5 nm for AMB, and overlain spectra was made. This is shown in Figures-1,2&3.

The stability study of DOX and AMB were performed by observing the absorbance of both at the concentration of 10 μg/ml at their wavelengths, at various time intervals 0 min, 10 min, 20 min, 30 min, 40 min, 50 min, 1 hr, 1 hr 15 min, 1 hr 30 min, 1 hr 45 min, 2 hr, 2 hr 30 min, 3 hr, 3 hr 30 min, 4 hr and 24 hr. The stability study of DOX and AMB are tabulated in Table-2. From the data shown, it was observed that DOX and AMB were stable in distilled water at their wavelengths.

6.1.1. Simultaneous equation method:

The individual and overlaid spectra of DOX and AMB were recorded as shown in Figure 1, 2 and 3. From the spectrums, 274 nm was λ_{max} of DOX and 244.5 nm was λ_{max} of AMB and these two wavelengths were used for the simultaneous estimation of DOX and AMB.

Different aliquots of DOX in distilled water were prepared in the concentration range of 7-35 μg ml^{-1}. The absorbances of these solutions were measured at 244.5 nm and 274 nm. The calibration curves were plotted using concentration against absorbance. The calibration graphs (mean value of six determinations) were plotted and are shown in Figures-4&5.

Different aliquots of AMB in distilled water were prepared in the concentration range of 1-5 μg ml^{-1}. The absorbances of solutions were measured at 244.5 nm and 274nm. The calibration curves were plotted using concentration against absorbance. The calibration graphs (mean value of six determinations) at 244.5 nm and 274 nm are shown in Figure-6&7. The optical parameters like Sandell's sensitivity, Molar absorptivity, Correlation coefficient, Slope, Intercept, LOD, LOQ and Standard error were calculated. The correlation coefficient for the two drugs was found to be about 0.999. This indicates that all the drugs obey Beer's law in the selected concentration range. Hence the curves were found to be linear. The optical characteristics of the two drugs at their selective wavelengths are shown in Table-3 for DOX and Table-4 for AMB.

The tablet containing DOX 400 mg and AMB 30 mg was selected for analysis. The nominal concentration of DOX from linearity i.e. 14 μg ml^{-1} was prepared and this contains 1 μg ml^{-1} concentration of AMB. The absorbance of the solution was measured at their respective wavelengths. The percentage label claim present in tablet formulation is given in Table-5 for DOX and AMB respectively.

The amount present in the tablet formulation was in good concord with the label claim and the % RSD values were found to be 0.0818 and 0.46779 for DOX and AMB respectively. The low % RSD values indicate that the method has good precision.

Further the precision of the method was confirmed by Intraday and Interday analysis. Analysis of the formulation was carried out for three times in the same day and one time in three consecutive days. The % RSD value of intraday and interday analysis were found to be 0.0638 and 0.0726 for DOX & 0.097 and 0.16348 for AMB. The results of the analysis are shown in Table-6. The results showed that the precision of the method was confirmed.

The developed method was validated for Ruggedness. It refers to the specific of one lab to multiple days which may include multiple Analysts, multiple instruments and different sources of reagents and so on. In the present work it was confirmed by different Analysts and different instruments. The % RSD value by Analyst 1 and Analyst 2 were found to be 0.09651 and 0.13017 for DOX & 0.23489 and 0.26049 for AMB respectively. The %RSD value by Instrument I and Instrument II were found to be 0.09929 and 0.0820 for DOX & 0.26661 and 0.46701 for AMB respectively. The low %RSD values indicate that the developed method was more rugged. The results are shown in Table- 7

The accuracy of the method was performed by recovery studies. To the preanalyzed formulation, a known quantity of DOX and AMB raw material solutions were added at different levels. The absorbance of the solutions was measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 99.98-100.087 % for DOX and 98.417-99.86% for AMB. The low % RSD value for the two drugs indicates that this method is very accurate. The recovery data is shown in Table-8. It indicates that there is no interference due to excipients present in the formulation. It can be easily and conveniently adopted for routine quality control analysis. This method is

accurate, simple, rapid, precise, reliable, sensitive, reproducible and economic, and is validated as per ICH guidelines.

6.1.2. Absorbance correction method

The individual and overlaid spectra of DOX and AMB were recorded and shown in Figure-1, 2 & 8. From the overlaid spectra, 308 nm was selected for the estimation of AMB without any interference from DOX, and 274 nm was selected for the estimation of DOX after the absorbance corrected for interference by AMB. The absorbance of DOX at 308 nm was zero and 274 nm was its λ_{max}.

Different aliquots of DOX and AMB were diluted to the concentration range separately in distilled water. The absorbance of each solution was measured at 274 nm and 308 nm. The calibration curve was plotted using absorbance against concentration. The calibration graphs at 274 nm and 308 nm for AMB is shown in Figures 5 & 9 and calibration graphs at 274 nm for DOX is shown in Figure-7. The preparation of calibration curve was repeated six times for each drug at their selective wavelengths. The optical parameters like Sandell's sensitivity, molar absorptivity, correlation coefficient, slope, intercept, LOD, LOQ and Standard error were calculated. The correlation coefficient for both the drugs were found to be about 0.999. This indicates that both the drugs obey Beer's law in the selected concentration range. Hence the concentrations were found to be linear. The optical characteristics of DOX and AMB at selected wavelengths are shown in Table-9 and Table-10 respectively.

The tablet containing DOX 400 mg and AMB 30 mg was selected for analysis. The nominal concentration of DOX from linearity i.e. 14 μg ml^{-1} (1 μg ml^{-1} of AMB) was prepared and the absorbance of the solutions were measured at their selected

wavelengths. The percentage label claim of the tablet formulation was found to be 100.32 ± 0.50529 for DOX and 99.60 ± 0.65582 for AMB. The amount present in the tablet formulation was in good concord with the label claim and the %RSD values were found to be 0.50529 and 0.65582 for DOX and AMB respectively. The low % RSD values indicate that the method has good precision. The result of formulation estimations is shown in Table-11.

Further the precision of the method was confirmed by intraday and interday studies. The %RSD values of intraday and interday analysis were found to be 0.16602 and 0.10613 for DOX & 0.58398 and 0.62845 for AMB. The results of analysis are shown in Table-12. The results showed that the precision of the method was high.

The developed method was validated for ruggedness. In the present work it was confirmed by different Analysts and different instruments. The % RSD value by Analyst 1 and 2 were found to be 0.13601 and 0.15235 for DOX & 0.72059 and 0.72059 for AMB. The % RSD value by Instrument 1 and 2 were found to be 0.09097 and 0.5051 for DOX & 0.65957 and 0.6550 for AMB. The low %RSD values indicate that the developed method was more rugged. The results are shown in Table-13.

The accuracy of the method was confirmed by recovery studies. To the pre-analyzed formulation, a known quantity of mixture of DOX and AMB raw material solutions were added at different levels. The absorbance of the solutions was measured at selected wavelengths and the percentage recovery was calculated. The percentage recovery was found to be in the range of 99.64-100 % for DOX and 99.73-100.55 % for AMB. The %RSD values were found to be less than 2 and this indicates that the method is accurate. The result of the recovery studies is shown in Table-14.

6.1.3. Absorbance ratio method

The individual and overlaid spectra of DOX and AMB were recorded and shown in Figures-1, 2 & 10. From the overlaid spectra, the wavelengths selected were 244.5 nm (λ_{max} of AMB) and 233.5 nm (iso-absorptive point).

The linearity of DOX and AMB was constructed in the range of 7-35 μg/ml and 1-5 μg/ml and their calibration curves are shown in Figures 11 & 12. The optical characteristics such as Beer's law limit (7-35 and 1-5 μg/ml), molar extinction coefficient, Sandell's sensitivity, correlation co-efficient, slope and intercept were calculated and are shown in Tables 15 & 16.

The amount present in the formulation was determined by calculating the average of six replicate analysis and its percentage purity was found to be in the range of 99.52-99.67% for DOX and 98.002-99.90% for AMB. The amount present in the tablet formulation was in good concord with the label claim and the %RSD values were found to be 0.05979 and 0.52331 for DOX and AMB respectively. The low %RSD values indicate that the method has good precision. The result of analysis is shown in Table-17.

Precision of the method was studied by making repeated analysis of the same sample and it was carried out three times in a day and for three days. The %RSD values of intraday and interday analysis were found to be 0.13374 and 0.12245 for DOX & 0.70482 and 0.66480 for AMB. The results of the analysis are shown in Table-18. The results showed that the precision of the method was high.

The developed method was validated for ruggedness. In the present work it was confirmed by different Analysts. The % RSD values for Analyst 1 and Analyst 2 were

found to be 0.10918 and 0.10595 for DOX & 0.67175 and 0.76009 for AMB. The % RSD value by Instrument 1 and 2 were found to be 0.10879 and 0.11213 for DOX & 0.77409 and 0.81303 for AMB. The low % RSD values indicate that the developed method was more rugged. The results are shown in Table-19.

The accuracy of the method was confirmed by recovery studies. The percentage recovery was found to be in the range of 100.30-100.50% for DOX and 99.00-99.83% for AMB. The %RSD values were found to be less than 2 and thus indicate that the method is accurate. The result of recovery study was shown in Table-20

6.2 REVERSE-PHASE HIGH PERFORMANCE LIQUID CHROMOTOGRAPHY FOR DOXOFYLLINE AND AMBROXOL HYDROCHLORIDE

An involvement was made in this project to device a simple, accurate, less expensive and sensitive RP-HPLC method of the estimation of DOX and AMB in solid dosage form. Since the drugs are polar, Reverse Phase High Performance Liquid Chromatography was selected.

Selection of mobile phase

The standard solutions containing DOX and AMB were injected into HPLC system and run in different solvent systems. By studying the literature survey, different mobile phases in different proportions and different pH were tried in order to find the best conditions for the separation.

Each mobile phase was sonicated for 10 minutes and filtered through 0.45 μ membrane filter. The mobile phase was allowed to equilibrate until steady baseline was obtained. The standard solutions containing DOX and AMB were run and combinations of solvents were tried to get a good separation and stable peak. From the various mobile phase tried,

mobile phase containing 10 mM Phosphate buffer: Acetonitrile: Methanol in the ratio of 70: 20: 10 (pH adjusted to 3.0 with orthophosphoric acid) was selected, since it gave sharp peak with symmetry and reproducible retention time for DOX and AMB.

Wavelength selection

The UV spectra of individual drugs were recorded in the wavelength range from 200-400 nm and compared. The choice to use a common wavelength set at 224 nm was considered satisfactory, permitting the detection of drugs with adequate sensitivity.

System suitability

The system suitability studies were carried out to determine Tailing factor, Asymmetrical factor, Theoretical plates and Capacity factor. The results are given in Table-21. The values obtained demonstrated the suitability of the system for the analysis of investigated drug combination and the system suitability parameters may fall within ±3% standard deviation range during routine performance of the method.

Stability

The stability of the drugs in the proposed mobile phase was checked by monitoring the absorbance of DOX and AMB at the selected wavelength over a period of 5 hours at room temperature. The result is reported in Table-22. The result shows that the absorbance of both the drugs remained almost unchanged and no significant degradation within the indicated period. Thus revealed that both the solutions were stable for at least 5 hours, which was sufficient to complete the whole analytical process.

Linearity

The linearity of the method was determined at five concentration levels ranging from 7-35 μg/ml for DOX and 0.5-2.5 μg/ml for AMB. The linearity chromatogram is recorded in Figures 13-17. The calibration curves were plotted between the mean peak areas vs. respective concentrations and are shown in Figures-18&19 for DOX and AMB respectively. The corresponding linear regression equation was y = 270225.0286 x + (-3.70062 E-09) with square of correlation coefficient r^2 of 0.999765561 for AMB and y = 169886.3316 x + (-17753.96429) with square of correlation coefficient r^2 of 0.999707379 for DOX respectively. The results showed that an excellent correlation exists between the peak area and concentration of the drugs within the concentration range indicated above and is represented in Table-23.

Quantification

The tablet dosage form containing DOX 400 mg and AMB 30 mg was selected for the analysis. The ostensible concentration 14 μg ml^{-1} of DOX in the mobile phase was prepared, which contains 1 μg ml^{-1} of AMB. 20 μl of each solution was injected and chromatograms were recorded and shown in Figures 20-25. The assay procedure was repeated for six times. The percentage purity was found to be 100.82% and 100.12 for DOX and AMB (Table-24). The result of analysis showed that the amount of drugs were in good agreement with the label claim of the formulation.

Method validation

The proposed HPLC method was validated as per the guidelines of the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human use (ICH).

The accuracy of the method was performed by recovery experiments. The recovery studies were carried out by standard addition method at three different levels 80%, 100% and 120% by injecting the solutions. The chromatograms are recorded as shown in the Figures 26-28. The percentage recovery was found to in the range between 98.36-99.76% for AMB and 98.54-99.43% for DOX. The low percentage of RSD values for recovery experiment indicates that the method is accurate. The values are given in the Table-25. The high percentage recovery revealed that there was no interference due to the excipients used in the formulation. Therefore the developed method was found to be accurate.

The precision of the method was demonstrated by interday and intraday variation studies. In the intraday study, six repeated injections of standard and sample solutions were made and % RSD was calculated. In the Interday variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and the % RSD was calculated. The results are presented in Table-26. From the data obtained, the developed HPLC method is found to be precise.

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

METOLAZONE AND SPIRONOLACTONE COMBINATION DOSAGE FORM:

The methods employed for the analysis of Metolazone and Spironolactone were

1. UV-Spectroscopic methods

a. Simultaneous equation method

b. Absorbance correction method and

c. Derivative spectroscopic method (First Order Derivative method)

2. Reverse-Phase High Performance Liquid Chromatography

6.3 UV-SPECTROSCOPIC STUDIES:

The solubility of MET and SPIR were determined in a variety of solvents as per ScHefter and Higuchi method[159]. 10 mg of samples were taken in a test tube and checked their solubility with variety of solvents as per IP and the profiles are shown in Table-27.

The numeral polar and non-polar solvents were attempted to dissolve the drugs. From the solubility profile, methanol followed by 0.02 M phosphate buffer (pH 3.5) was chosen as a solvent for the estimation of MET and SPIR in bulk and in formulation.

Three accurate, simple and rapid UV methods namely Simultaneous equation method, Absorbance correction method and First order derivative spectroscopy method were selected.

The drugs were dissolved in methanol followed by 0.02 M phosphate buffer, pH 3.5 to produce 10 μg/ml. Scanned in the UV-region of 200-400 nm, it shows constant wavelength at 236.5 nm for MET and 242.5 nm for SPIR, and overlain spectra was made. This is shown in Figures-29,30&31.

The stability study of MET and SPIR was performed by observing the absorbance of both drugs at the concentration of 10 μg/ml at their wavelengths, at various time intervals 0 min, 10 min, 20 min, 30 min, 40 min, 50 min, 1 hr, 1 hr 15 min, 1 hr 30 min, 1 hr 45 min, 2 hr, 2 hr 30 min, 3 hr, 3 hr 30 min, 4 hr, 5 hr and 24 hr. The result of the stability study of MET and SPIR is tabulated in Table-28. From the data shown, it was observed that MET and SPIR were stable in methanol followed by 0.02 M phosphate buffer, pH 3.5 at their wavelengths.

6.3.1 Simultaneous equation method:

The individual and overlaid spectra of MET and SPIR were recorded as shown in Figures 29, 30 & 31. From the spectrums, 236.5 nm was λ_{max} of MET and 242.5 nm was λ_{max} of SPIR. These two wavelengths were used for the simultaneous estimation of MET and SPIR.

Different aliquots of MET in methanol followed by 0.02 M phosphate buffer pH 3.5 were prepared in the concentration range of 0.5-2.5 μg ml^{-1}. The absorbances of solutions were measured at 236.5 nm and 242.5 nm. The calibration curve was plotted using concentration against absorbance. The calibration graphs at 236.5 nm and 242.5 nm are shown in Figure-32&33.

Different aliquots of SPIR in methanol followed by phosphate buffer pH 3.5 were prepared in the concentration range of 5-25 μg ml^{-1}. The absorbances of these solutions were measured at 236.5 nm and 242.5 nm. The calibration graphs were plotted and are shown in Figures 34 & 35. The preparation of calibration curve was repeated six times for each drug at their selective wavelengths. The optical parameters like Sandell's

sensitivity, Molar absorptivity, Correlation coefficient, Slope, Intercept, LOD, LOQ and Standard error were calculated. The correlation coefficient for the two drugs was found to be about 0.999. This indicates that all the drugs obey Beer's law in the selected concentration range. Hence the curves were found to be linear. The optical characteristics of two drugs at their selective wavelengths are shown in Table-29 for MET and Table-30 for SPIR.

The tablet (METOLACTONE-5) containing Metolazone and Spironolactone was selected for analysis. The nominal concentration of MET from linearity (1 μg ml^{-1}) was prepared and also contains (10 μg ml^{-1}) SPIR. The absorbances of the solution were measured at their respective wavelengths. The percentage purity of the drugs present in tablet formulation is given in Table-31 for MET and SPIR.

The amount present in tablet formulation was in good concord with the label claim and the %RSD values were found to be 1.15105 and 0.22899 for MET and SPIR, respectively. The %RSD values were found to be less than 2, which indicate 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 three consecutive days. The %RSD values of intraday and interday analysis were found to be 1.04542 and 1.05537 for MET & 0.215447 and 1.05537 for SPIR. The results of analysis are shown in Table-32. The results show that the precision of the method is confirmed.

The developed method was validated for Ruggedness. In the present work, it was confirmed by different Analysts and different instruments. The %RSD value for Analyst 1 and Analyst 2 were found to be 0.84257 and 0.88495 for MET & 0.21753 and 0.16584 for SPIR respectively. The %RSD value for Instrument I and Instrument II were found to 0.83696 and 0.87376 for MET & 0.14684 and 0.13877 for SPIR. The low %RSD values indicate that the developed method is more rugged. The results are shown in Table-33.

The accuracy of the method was performed by recovery studies. To the preanalyzed formulation, a known quantity of MET and SPIR raw material solutions were added at different levels. The absorbances of the solutions were measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 99.67-100.27 % for MET and 99.41-101.36% for SPIR. The low %RSD values for the two drugs indicate that this method is very accurate. The recovery data is shown in Table-34.

The result indicates that there is no interference due to excipients present in the formulation. The method can be easily and conveniently adopted for routine quality control analysis. And also this method is accurate, simple, rapid, precise, reliable, sensitive, reproducible & economic, and it is validated as per ICH guidelines.

6.3.2. Absorbance Correction Method:

From the overlaid spectrum of MET and SPIR, 345 nm was selected for the estimation of MET without any interference from SPIR and 242.5 nm was selected for the estimation of SPIR after the absorbance corrected for interference by MET. The absorbance of SPIR at 345 nm is zero and 242.5 nm is its λ_{max}. This is shown in Figure-36.

Different aliquots of MET in methanol followed by 0.02 M phosphate buffer, pH 3.5 were prepared in the concentration range of 0.5-2.5 μg ml^{-1}. The absorbances of the solutions were measured at 242.5 nm and 345 nm. The calibration curve (mean value of six determinations) was plotted using concentration against absorbance. The calibration graphs at 242.5 nm and 345 nm are shown in Figures-33&37.

Different aliquots of SPIR in methanol followed by phosphate buffer, pH 3.5 were prepared in the concentration range of 5-25 μg ml^{-1}. The absorbances of these solutions were measured at 242.5 nm and 345 nm. The calibration graph (mean value of n=6) at 242.5 nm is plotted and is shown in Figure 35. The preparation of calibration curve repeated six times for each drug at their selective wavelengths. The optical parameters like Sandell's sensitivity, Molar absorptivity, Correlation coefficient, Slope, Intercept, LOD, LOQ and Standard error were calculated. The correlation coefficient for the two drugs was found to be about 0.999. This indicates that the drugs obey Beer's law in the selected concentration range. Hence the curves were found to be linear. The optical characteristics of two drugs at their selective wavelengths are shown in Table-35 for MET and Table-36 for SPIR.

The tablet formulation (METOLACTONE-5) containing Metolazone 5 mg and Spironolactone 50 mg was selected for analysis. The nominal concentration of MET from linearity (1 μg ml^{-1}) was prepared and also contains 10 μg ml^{-1} of SPIR. The absorbances of the solutions were measured at their respective wavelengths. The percentage purity of the drugs present in the tablet formulation was given in Table-37 for MET and SPIR.

The amount present in tablet formulation was in good concord with the label claim and the %RSD values were found to be 0.95688 and 0.18878 for MET and SPIR respectively.

The %RSD values were found to be less than 2, which indicate that the method has good precision.

Further the precision of the method was confirmed by Intraday and Interday analysis. The %RSD value of intraday and interday analysis were found to be 0.87397 and 0.80393 for MET & 0.20075 and 0.21296 for SPIR. The results of analysis are shown in Table-38. The results show that the precision of the method is confirmed.

The developed method was validated for Ruggedness. In the present work, Ruggedness was confirmed by different Analysts and different instruments. The %RSD value for Analyst 1 was found to be 0.62358 for MET & 0.29421 for SPIR. For Analyst 2, it was 0.62358 for MET and 0.24676 for SPIR. The %RSD value for Instrument I and Instrument II were found to be 0.58440 and 0.57106 for MET & 0.31223 and 0.30181 for SPIR. The low %RSD values indicate that the developed method is more rugged. The results are shown in Table-39.

The accuracy of the method was performed by recovery studies. To the preanalyzed formulation, a known quantity of MET and SPIR raw material solutions were added at 80%, 100% and 120%. The absorbances of the solutions were measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 99.95-100.29 % for MET and 100.52-100.63% for SPIR. The low % RSD value for the two drugs indicates that this method is very accurate. The recovery data is shown in Table-40. The result indicates that there is no interference due to excipients present in the formulation. It can be easily and conveniently adopted for routine quality control analysis. This method is accurate, simple, rapid, precise, reliable, sensitive, reproducible and economic, and is validated as per ICH guidelines.

6.3.3. Derivative Spectroscopic Method

A simple, accurate, rapid and precise first order derivative method was developed and validated. The solvent selected for the estimation of Metolazone and Spironolactone is methanol followed by 0.02M phosphate buffer pH 3.5, adjusted with phosphoric acid.

The sample solutions of 10 μg ml^{-1} of MET and 100 μg ml^{-1} of SPIR in the corresponding solvent was prepared and the solutions were scanned in the UV region of 200 to 400 nm by using methanol followed by 0.02 M phosphate buffer, pH 3.5, adjusted with phosphoric acid as blank. The zero order spectrums were derivatised into first order derivative spectrums. The first order derivative spectrums of MET, SPIR and their overlaid spectrum were recorded and are shown in Figures 38, 39 & 40. From the spectrum, 289 nm and 266 nm were selected for the estimation of MET and SPIR respectively without any interference. At 289 nm, the absorbance of SPIR was zero. At 266 nm, the absorbance of MET was zero. Hence these two wavelengths were selected for the analysis of MET and SPIR.

Different aliquots of MET and SPIR were prepared in the concentration range of 1-5 μg ml^{-1} and 10-50 μg ml^{-1} respectively. The absorbances of the solutions were measured at 266 nm and 289 nm in the first order derivative spectrum for MET and SPIR respectively. The plotted graphs for MET and SPIR are shown in Figures 41 & 42. The preparation of calibration curve was repeated six times for each drug at their selective wavelength. The calibration curve was plotted using concentration against absorbance. The optical parameters like Sandell's sensitivity, Molar absorptivity, Correlation coefficient, Slope, Intercept, LOD, LOQ and Standard error were calculated for the two drugs. The correlation coefficient for the two drugs was found to be about 0.999. This

indicates that the two drugs obey Beer's law in the selected concentration range. Hence the concentrations were found to be linear. The results are shown in Table-41.

METOLACTONE-5 containing Metolazone 5 mg and Spironolactone 50 mg was selected for analysis. The solution contains 3 μg ml^{-1} of MET was prepared (nominal concentration in the calibration curve of MET), which also contains 30 μg ml^{-1} of SPIR. The absorbances of the solutions were measured at 266 nm and 289 nm and the amount of six test solutions were determined. The percentage purity present in tablet formulation was found to be 100.20 ± 1.27495 and 99.97 ± 0.31009 for MET and SPIR respectively. The amount present in tablet formulation was in good concord with the label claim and the % RSD values were found to be less than 2. Hence the method has good precision. The results of analysis are shown in Table-42.

Further the precision of the method was confirmed by Intraday and Interday analysis. The % RSD value of Intraday and Interday analysis are 1.35431 and 1.32968 for MET & 0.30948 and 0.58321 for SPIR. The results of analysis are shown in Table-43. The result shows that the precision of the method is confirmed.

The developed method was validated for Ruggedness. In the present work it was confirmed by different Analysts and different instruments. The % RSD values for Analyst 1 and Analyst 2 wer found to be 1.61435 and 1.46093 for MET and 0.39317 and 0.29887 for SPIR. The % RSD values for Instrument 1 and Instrument 2 wer found to be 1.21345 and 1.32123 for MET and 0.67843 and 0.72357 for SPIR.The %RSD values were found to be less than 2, which indicate that the developed method is more rugged. The results are shown in Table-44.

The accuracy of the method was performed by recovery studies. To the pre-analyzed formulation, a known quantity of MET and SPIR raw material solutions were added at different levels. The absorbance of the solutions was measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 99.81-100.92 % for MET and 100.16-100.31 % for SPIR. The %RSD values for the two drugs were found to be less than 2 and it indicates that this method is very accurate. The recovery data is shown in Table-45.

6.4 REVERSE-PHASE HIGH PERFORMANCE LIQUID CHROMOTOGRAPHY FOR METOLAZONE AND SPIRONOLACTONE:

The reverse phase HPLC was selected for separation because it is convenient and rugged than other forms of the liquid chromatography and is more likely to result in a satisfactory final separation.

Selection of mobile phase

This work was focused on optimization of the conditions for the simple and rapid as well as low cost effective analysis including a selection of the proper column and mobile phase to obtain satisfactory results. To optimize the RP-HPLC parameters, several mobile phase compositions were tried. Taking into consideration of the system suitability parameters like retention time, peak symmetry and number of theoretical plates, the mobile phase found to be the most suitable for analysis was 25 mM phosphate buffer (pH 3.5): acetonitrile: methanol in the ratio of 40: 30: 30. The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1 ml/min. The proposed chromatographic conditions were found to be appropriate for the quantitative determination.

Wavelength selection

The λ_{max} of MET was 236.5 nm and for SPIR it was 243 nm in the mobile phase. The isoabsorptive point for both drugs was found to be 238 nm. So it was selected as detection wavelength.

System suitability

The system suitability parameters such as Theoretical plate, Tailing factor, Asymmetric factor (<2) and Capacity factor were calculated and shown in Table-46. The parameters were found to be satisfactory as per ICH guidelines.

Stability

The stability of the drugs in the mobile phase was checked by monitoring the absorbance of MET and SPIR at the specified wavelength over a period of 2 hours 30 min at room temperature. The results are reported in Table-47. The results show that the absorbance of both the drugs remained almost unchanged and no significant degradation within the indicated period and thus reveals that both solutions were stable for at least 2 hours 30 min, which was sufficient to complete the whole analytical process.

Linearity

To establish the linearity of the analytical method, a series of dilution ranging from 0.5-2.5 µg/ml for MET and 5-25 µg/ml for SPIR was prepared. All the solutions were filtered through 0.22µ membrane filter and injected, as well as the chromatograms were recorded (Figures 43-47). The calibration graphs were plotted between the mean peak area vs. respective concentration and are shown in Figures 48 & 49. The corresponding linear regression equation was y = 1745969.295 x + 32452.1587 with square of correlation

coefficient r^2 of 0.9998 for MET and y = 169886.3316 x + (-17753.96429) with square of correlation coefficient r^2 of 0.9997 for SPIR. The optical characteristics of MET and SPIR are represented in Table-48. The results show that there exists an excellent correlation between the peak area and concentration of drugs within the concentration range indicated above.

Quantification

The tablet formulation (METOLACTONE-5) containing Metolazone 5 mg and Spironolactone 50 mg was selected for the analysis. The ostensible concentration 1 μg ml^{-1} of MET was prepared which contains 10 μg ml^{-1} of SPIR. 20 μl of each solution was injected and chromatograms were recorded and are shown in Figures 50-55. The assay procedure was repeated for six times. The percentage purity was found to be 99.23 % for MET and 100.29 % for SPIR (Table-49). The results of analysis show that the amount of drugs was in good agreement with the label claim of the formulation.

Method validation:

The proposed HPLC method was validated as per the guidelines of the International conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human use (ICH).

The accuracy of the method was performed by recovery experiments. The recovery studies were carried out by standard addition method at three different levels 80%, 100% and 120% by injecting the solutions. The chromatograms were recorded as shown in the Figures 56-58. The percentage recovery was found to in the range between 99.74-

100.33% for MET and 99.93-101.08% for SPIR. The low %RSD values for recovery studies indicate that the method is accurate. The values are given in the Table-50.

The precision of the method was demonstrated by interday and intraday variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and %RSD was calculated. In the interday variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and the %RSD was calculated. The results are represented in Table-51. From the data obtained, the developed HPLC method was found to be precise.

These data show that the proposed method is sensitive for the determination of MET and SPIR. It was observed that there is no interference of the excipients with the principal peak. Hence the method is specific for the estimation of Metolazone and Spironolactone.

METOPROLOL AND OLMESARTAN COMBINATION DOSAGE FORM:

The UV-Spectroscopic methods employed for the analysis of Metoprolol and Olmesartan were

a. Simultaneous Equation Method

b. Area Under the Curve Method and

c. Derivative Spectroscopic Method

6.5 UV-SPECTROSCOPIC STUDIES:

The solubility of Metoprolol and Olmesartan were determined in a variety of solvents as per ScHefter and Higuchi method[159]. 10 mg of samples were taken in test tube and checked their solubility with variety of solvents as per IP and the profiles were shown in Table-52.

The numeral polar and non-polar solvents were attempted to dissolve the drugs. From the solubility profile, methanol was chosen as a solvent for the estimation of METO and OLME in bulk and formulation.

Three accurate, simple and rapid UV methods namely Simultaneous equation method, Area under the curve method and First order derivative spectroscopy method were selected.

The drugs were dissolved in methanol to produce 10 µg/ml. Scanned in the UV-region of 200-400 nm by using methanol as blank, it shows constant wavelength at 223.5 nm for METO and 256.5 nm for OLME. The overlaid spectrum was also made. These are shown in Figures 59, 60 & 61.

The stability study of METO and OLME was performed by observing the absorbance of both at the concentration of 10 µg/ml at their wavelengths, at various time intervals 0 min, 10 min, 20 min, 30 min, 40 min, 50 min, 1 hr, 1 hr 15 min, 1 hr 30 min, 2 hr, 2 hr 30 min, 3 hr, 4 hr, 5 hr and 24 hr. The stability study of METO and OLME are tabulated in Table-53. From the data shown, it was observed that METO and OLME were stable in methanol at their wavelengths.

6.5.1. Simultaneous Equation Method:

The individual and overlaid spectra of METO and OLME were recorded as shown in Figures 59, 60 & 61. From the spectrum, 223.5 nm was selected as λ_{max} of METO and 256.5 nm was λ_{max} of OLME. These two wavelengths were used for the simultaneous estimation of METO and OLME.

Different aliquots of METO in methanol were prepared in the concentration range of 5-25 μg ml^{-1}. The absorbances of the solutions were measured at 223.5 nm and 256.5 nm. The calibration curve (mean value of six determinations) was plotted using concentration against absorbance. The calibration graphs at 223.5 nm and 256.5 nm are shown in Figures-62 & 63.

Different aliquots of OLME in methanol were prepared in the concentration range of 4-20 μg ml^{-1}. The absorbances of these solutions were measured at 223.5 nm and 256.5 nm. The calibration graphs (mean value of six determinations) were plotted and are shown in 7Figures- 64 & 65. The preparation of calibration curve was repeated six times for each drug at their selective wavelengths. The optical parameters like Sandell's sensitivity, Molar absorptivity, Correlation coefficient, Slope, Intercept, LOD, LOQ and Standard error were calculated. The correlation coefficient for the two drugs was found to be about 0.999. This indicates that all the drugs obey Beer's law in the selected concentration range. Hence the curves were found to be linear. The optical characteristics of two drugs at their selective wavelengths are shown in Table-54 for METO and Table-55 for OLME.

The capsule formulation (OLSAR-M 25) and tablet formulations (OLMESAR-M & OLMAX-M) were selected for analysis. OLSAR-M 25 and OLMESAR-M contain METO 25 mg and OLME 20 mg. OLMAX-M contains METO 50 mg and OLME 20 mg.

In OLSAR-M 25 and OLMESAR-M, the nominal concentration of METO 10 μg ml^{-1} from linearity was prepared and also contains 8 μg ml^{-1} OLME. In OLMAX-M, the nominal concentration of METO 20 μg ml^{-1} from linearity was prepared, which contains 8 μg ml^{-1} OLME. The absorbances of the solutions were measured at their respective

wavelengths. The percentage purity present in the formulations are given in Tables-56, 57 & 58.

The amount present in the formulations were in good concord with the label claim and the %RSD values for OLSAR-M 25 was found to be 0.18167 and 0.10070 for METO and OLME respectively. For OLMESAR-M, % RSD value was 0.54152 and 0.35692. For OLMAX-M it was found to be 0.36097 and 0.64287. The low %RSD values indicate that the method has good precision.

Further the precision of the method was confirmed by Intraday and Interday analysis. The analysis of the formulations were carried out for three times in the same day and one time in three consecutive days. In OLSAR-M 25, the % RSD value of intraday and interday analysis were found to be 0.10261 and 0.15865 for METO & 0.07444 and 0.06500 for OLME. In OLMESAR-M, the % RSD value of intraday and interday analysis were found to be 0.38916 and 0.18969 for METO & 0.16057 and 0.80851 for OLME. In OLMAX-M, the % RSD value of intraday and interday analysis were found to be 0.13605 and 0.06523 for METO & 0.18043 and 0.18427 for OLME. The results of analysis are shown in Table-59, 60 & 61. The results show that the precision of the method is confirmed and comparatively OLMAX-M formulation show high precision.

The developed method was validated for Ruggedness. In the present work it was confirmed by different Analysts and different instruments. In OLSAR-M 25, the % RSD value for Analyst 1 and Analyst 2 were found to be 0.13246 and 0.10628 for METO & 0.05886 and 0.11211 for OLME., and the % RSD values for Instrument I and Instrument II were found to be 0.15462 and 0.18200 for METO & 0.05197 and 0.10000 for OLME.

In OLMESAR-M, the % RSD value for Analyst 1 and Analyst 2 were found to be 0.20876 and 0.26573 for METO & 0.10403 and 0.17920 for OLME., and the % RSD value for Instrument I and Instrument II were found to be 0.19092 and 0.15824 for METO & 0.09008 and 0.07116 for OLME.

In OLMAX-M, the % RSD value for Analyst 1 and Analyst 2 were found to be 0.13301 and 0.10975 for METO & 0.09239 and 0.13875 for OLME., and the % RSD value for Instrument I and Instrument II were found to be 0.19727 and 0.05176 for METO & 0.07113 and 0.07404 for OLME.

The low %RSD values for all formulations indicate that the developed method is more rugged. The results are shown in Tables-62,63&64.

The accuracy of the method was performed by recovery studies. To the preanalyzed formulations, a known quantity of METO and OLME raw material solutions were added at different levels. The absorbance of the solutions was measured and the percentage recovery was calculated for all formulations. In OLSAR-M 25, the percentage recovery was found to be in the range of 99.98-100.48% for METO and 100.29-101.08% for OLME. In OLMESAR-M, the percentage recovery was found to be in the range of 99.59-100.51% for METO and 100.34-101.23% for OLME. In OLMAX-M, the percentage recovery was found to be in the range of 100.07-100.16% for METO and 99.70-101.30% for OLME.

The low %RSD values for all formulations indicate that this method is very accurate. The recovery data is shown in Tables-65, 66 &67. It indicates that there is no interference due to excipients present in the formulations. It can be easily and conveniently adopted for

routine quality control analysis. This method is accurate, simple, rapid, precise, reliable, sensitive, reproducible & economic, and is validated as per ICH guidelines.

6.5.2. Area under the curve method:

For the selection of wavelength, a suitable standard solutions containing 10 μg ml^{-1} of METO and OLME were prepared individually and scanned in the entire range from 200-400 nm, and overlaid spectrum was made.

From the overlaid spectrum, areas were measured at wavelengths between 218-228 nm and 246-266 nm for the determination of METO and OLME respectively as shown in Figure-66.

Different aliquots of METO in methanol were prepared in the concentration range of 5-25 μg ml^{-1} and scanned in the UV region of 200-400 nm. The areas were measured at wavelengths 218-228 nm and 246-266 nm. The calibration curve was plotted using concentration against area at specified wavelengths. The calibration graphs (mean value) at 218-228 nm and 246-266 nm are shown in Figures-67&68.

Different aliquots of OLME in methanol were prepared in the concentration range of 4-20 μg ml^{-1} and scanned in the UV region of 200-400 nm. The areas were measured at wavelengths 218-228 nm and 246-266 nm. The calibration curve was plotted using concentration against area at specified wavelengths. The calibration graphs (mean value) were plotted and are shown in Figures-69&70.

The preparation of calibration curve was repeated six times for each drug at their specified wavelength region. The optical parameters like Sandell's sensitivity, Molar absorptivity, Correlation coefficient, Slope, Intercept, LOD, LOQ and Standard error

were calculated. The correlation coefficient for the two drugs was found to be about 0.999. This indicates that all the drugs obey Beer's law in the selected concentration range. Hence the curves were found to be linear. The optical characteristics of two drugs at their selective wavelengths are shown in Table-68 for METO and Table-69 for OLME.

The capsule formulation (OLSAR-M 25) and tablet formulations (OLMESAR-M & OLMAX-M) were selected for analysis. In OLSAR-M 25 and OLMESAR-M, the nominal concentration of METO 10 μg/ml from linearity was prepared and also contains 8 μg ml^{-1} OLME. In OLMAX-M, the nominal concentration of METO 20 μg ml^{-1} from linearity was prepared, which contains 8 μg ml^{-1} OLME. The areas of samples were measured between 218-228 nm and 246-266 nm. The percentage purity present in the formulations were given in Tables-70,71&72.

The amount present in the formulations was in good concord with the label claim. The %RSD values for OLSAR-M 25 was found to be 0.18127 and 0.02585 for METO and OLME respectively. For OLMESAR-M, % RSD value was 0.23800 and 0.06847. For OLMAX-M it was found to be 0.21357 and 0.09845. The low %RSD values indicate that the method has good precision.

Further the precision of the method was confirmed by Intraday and Interday analysis. In OLSAR-M 25, the % RSD value of intraday and interday analysis were found to be 0.18574 and 0.51806 for METO 0.05886 & 0.37368 and for OLME. In OLMESAR-M, the % RSD value of intraday and interday analysis were found to be 0.36490 and 0.56088 for METO & 0.32437 and 0.27801 for OLME. In OLMAX-M, the % RSD value of intraday and interday analysis were found to be 0.16013 and 0.14347 for METO &

0.23089 and 0.18858 for OLME. The results of analysis are shown in Tables-73, 74 &74. The results show that the precision of the method is confirmed.

The developed method was validated for Ruggedness. In the present work, ruggedness was confirmed by different Analysts and different instruments. In OLSAR-M 25, the %RSD values for Analyst 1 and Analyst 2 were found to be 0.20177 and 0.37615 for METO & 0.03766 and 0.06897 for OLME, and the % RSD values for Instrument I and Instrument II were found to be 0.1540 and 0.1820 for METO & 0.0520 and 0.10000 for OLME.

In OLMESAR-M, the % RSD value for Analyst 1 and Analyst 2 were found to be 0.30905 and 0.13725 for METO & 0.21663 and 0.20275 for OLME, and the % RSD value for Instrument I and Instrument II were found to be 0.29563 and 0.29563 for METO & 0.10817 and 0.12421 for OLME.

In OLMAX-M, the % RSD value for Analyst 1 and Analyst 2 were found to be 0.00163 and 0.09180 for METO & 0.26647 and 0.30723 for OLME, and the % RSD value for Instrument I and Instrument II were found to be 0.11443 and 0.12634 for METO & 0.36663 and 0.37162 for OLME. The low %RSD values for all formulations indicate that the developed method is more rugged. The results are shown in Tables-76, 77 & 78.

The accuracy of the method was performed by recovery studies. To the preanalyzed formulation, a known quantity of METO and OLME raw material solutions were added at 80%, 100% and 120%. The areas of samples were measured between 218-228 nm and 246-266 nm, and the percentage recovery was calculated.

In OLSAR-M 25, the percentage recovery was found to be in the range of 99.78-100.92% for METO and 99.89-100.17% for OLME. In OLMESAR-M, the percentage recovery was found to be in the range of 99.32-100.37% for METO and 98.75-100.32% for OLME. In OLMAX-M, the percentage recovery was found to be in the range of 100.12-100.15% for METO and 100.05-100.20% for OLME. The low %RSD values of two drugs in all the formulations indicate that this method is very accurate. The recovery datas are shown in Tables-79, 80 & 81. It indicates that there is no interference due to excipients present in the formulations. It can be easily and conveniently adopted for routine quality control analysis. This method is accurate, simple, rapid, precise, reliable, sensitive, reproducible & economic and it is validated as per ICH guidelines.

6.5.3. Derivative Spectroscopic Method

A simple, accurate, rapid and precise first order derivative method was developed and validated. The solvent selected for the estimation of METO and OLME is methanol.

The sample solutions of 10 μg/ml of METO and OLME in methanol were prepared and the solutions were scanned in the UV region in the wavelength range from 200 to 400 nm by using methanol as blank. The zero order spectrums were derivatised into first order derivative spectrum. The first order derivative spectrums of METO and OLME and their overlaid spectrum were recorded and shown in Figures 71, 72 & 73. From the spectrums, 256 nm and 243 nm were selected for the estimation of METO and OLME respectively without any interference. At 256 nm, the absorbance of OLME was zero. At 243 nm, the absorbance of METO was zero. Hence these two wavelengths were selected for the analysis of METO and OLME

Different aliquots of METO and OLME were prepared in the concentration range of 5-25 μg/ml and 4-20 μg/ml respectively. The absorbances of these solutions were measured at 256 nm and 243 nm in the first order derivative spectrum for METO and OLME respectively. The plotted graphs for METO and OLME are shown in Figures 74 & 75. The preparation of calibration curve was repeated six times for each drug at their selective wavelength. The calibration curve was plotted using concentration against absorbance. The optical parameters like Sandell's sensitivity, Molar absorptivity, Correlation coefficient, Slope, Intercept, LOD, LOQ and Standard error were calculated for the two drugs. The correlation coefficient for the two drugs was found to be about 0.999. This indicates that the two drugs obey Beer's law in the selected concentration range. Hence the concentrations were found to be linear. The results are shown in Table-82.

The capsule formulation (OLSAR-M 25) and tablet formulations (OLMESAR-M & OLMAX-M) were selected for analysis. In OLSAR-M 25 and OLMESAR-M, the solutions containing 10 μg/ml of METO was prepared (nominal concentration in the calibration curve of METO), which also contains 8 μg/ml of OLME. In OLMAX-M, the solution containing 20 μg/ml of METO was prepared which contains 8 μg/m of OLME. The absorbance of the solutions was measured at 256 nm and 243 nm. The amount of the test solutions was determined.

In OLSAR-M 25, the percentage purity of drugs was found to be 99.96% and 100.06% for METO and OLME respectively. In OLMESAR-M, the percentage purity of drugs was found to be 99.81% and 100.00% for METO and OLME respectively. In OLMAX-M, the

percentage purity of drugs was found to be 100.51% and 100.15% for METO and OLME respectively.

The amount present in METO and OLME in all the formulations was in good concord with the label claim and the % RSD values were found to be less than 2. Hence the method has good precision. The results of analysis are shown in Tables-83,84&85.

Further the precision of the method was confirmed by Intraday and Interday analysis. In OLSAR-M 25, the %RSD value of Intraday and Interday analysis are 0.76436 and 0.72234 for METO & 0.50339 and 0.61508 for OLME. In OLMESAR-M, the % RSD value of intraday and interday analysis were found to be 0.73556 and 0.74819 for METO & 0.62117 and 0.4724 for OLME. In OLMAX-M, the % RSD value of intraday and interday analysis were found to be 0.14404 and 0.32905 for METO & 0.35659 and 0.45456 for OLME. The results of analysis are shown in Tables- 86, 87 & 88. The results show that the precision of the method is confirmed and comparatively OLMAX-M show high precision.

The developed method was validated for Ruggedness. In the present work it was confirmed by different Analysts and different instruments. In OLSAR-M 25, the %RSD values for both Analyst 1 and Analyst 2 were found to be 0.85257 for METO and for OLME, the % RSD values for Analyst 1 and Analyst 2 were found to be 0.61125 and 0.67588 respectively. The % RSD value for Instrument I and Instrument II were found to be 0.87531 and 0.86756 for METO & 0.73458 and 0.76783 for OLME.

In OLMESAR-M, the % RSD value for Analyst 1 and Analyst 2 were found to be 0.32087 and 0.31728 for METO & 0.28690 and 0.2966 for OLME, and the % RSD value

for Instrument I and Instrument II were found to be 0.80028 and 0.67820 for METO & 0.05263 and 0.05170 for OLME.

In OLMAX-M, the % RSD value for Analyst 1 and Analyst 2 were found to be 0.06524 and 0.05207 for METO & 0.31389 and 0.2090 for OLME, and the % RSD value for Instrument I and Instrument II were found to be 0.06625 and 0.08064 for METO & 0.26233 and 0.19384 for OLME. The low %RSD values for all formulations indicate that the developed method is more rugged. The results are shown in Tables-89, 90 & 91. The % RSD values were found to be less than 2, which indicate that the developed method is more rugged.

The accuracy of the method was performed by recovery studies. To the preanalyzed formulations, a known quantity of METO and OLME raw material solutions were added at different levels. The absorbances of the solutions were measured and the percentage recovery was calculated.

In OLSAR-M 25, the percentage recovery was found to be in the range of 100.21-100.47% for METO and 100.21-100.47 % for OLME. In OLMESAR-M, the percentage recovery was found to be in the range of 100.64-100.76 % for METO and 99.73-99.92% for OLME. In OLMAX-M, the percentage recovery was found to be in the range of 100.03-100.05% for METO and 99.95-100.25 for OLME.

The % RSD values of the drugs in all the formulations were found to be less than 2 and this indicates that this method is very accurate. The recovery datas are shown in Tables-92, 93 & 94.

ASPIRIN AND ROSUVASTATIN COMBINATION DOSAGE FORM:

The methods employed for the analysis of Aspirin and Rosuvastatin were

1. UV-Spectroscopic methods

a. Simultaneous equation method and

b. Absorbance ratio method and

6.6 UV-SPECTROSCOPIC STUDIES:

The solubility of ASP and ROSU were determined in a variety of solvents as per ScHefter and Higuchi method[159]. 10 mg of samples were taken in a test tube and checked their solubility with variety of solvents as per IP and the profiles are shown in Table-95.

The numeral polar and non-polar solvents were attempted to dissolve the drugs. From the solubility profile, methanol was chosen as a solvent for the estimation of ASP and ROSU in bulk and in formulation.

The accurate, simple and rapid UV methods namely Simultaneous equation method and Absorbance ratio method were selected.

Aspirin and Rosuvastatin were dissolved in methanol to produce 10 µg/ml and it was scanned in the UV-region of 200-400 nm. UV spectrum of individual drugs shows constant wavelength at 294.5 nm for ASP and 243 nm for ROSU. The overlain spectrum was also made. This is shown in Figures 76, 77 & 78.

The stability study of ASP and ROSU was performed by observing the absorbance of both drugs at the concentration of 10 µg/ml at their wavelengths, at various time intervals 0 min, 10 min, 20 min, 30 min, 40 min, 50 min, 1 hr, 1 hr 15 min, 1 hr 30 min, 1 hr 45

min, 2 hr, 2 hr 30 min, 3 hr, 3 hr 30 min, 4 hr and 24 hr. The result of the stability study of ASP and ROSU was tabulated in Table-96. From the data shown, it was observed that ASP and ROSU were stable in methanol at their wavelengths.

6.6.1 Simultaneous equation method:

The individual and overlaid spectra of ASP and ROSU were recorded as shown in Figures 76, 77 & 78. From the spectrums, 294.5 nm was λ_{max} of ASP and 243 nm was λ_{max} of ROSU. These two wavelengths were used for the simultaneous estimation of ASP and ROSU.

Different aliquots of ASP in methanol were prepared in the concentration range of 7.5-37.5 $\mu g\ ml^{-1}$. The absorbances of solutions were measured at 294.5 nm and 243 nm. The calibration curve was plotted using concentration against absorbance. The calibration graphs at 294.5 nm and 243 nm are shown in Figures- 79 & 80.

Different aliquots of ROSU in methanol were prepared in the concentration range of 1-5 $\mu g\ ml^{-1}$. The absorbances of these solutions were measured at 294.5 nm and 243 nm. The calibration graphs were plotted and are shown in Figures 81 & 82. The preparation of calibration curve was repeated six times for each drug at their selective wavelengths. The optical parameters like Sandell's sensitivity, Molar absorptivity, Correlation coefficient, Slope, Intercept, LOD, LOQ and Standard error were calculated. The correlation coefficient for the two drugs was found to be about 0.999. This indicates that ASP and ROSU obey Beer's law in the selected concentration range. Hence the curves were found to be linear. The optical characteristics of the two drugs at their selective wavelengths are shown in Table-97 for ASP and Table-98 for ROSU.

The capsule (**ROZUCOR ASP-10)** containing Aspirin 75 mg and Rosuvastatin 10 mg was selected for analysis. The nominal concentration of ASP from linearity (15 μg ml^{-1}) was prepared and also contains (2 μg ml^{-1}) ROSU. The absorbances of the solutions were measured at their respective wavelengths. The percentage purity of the drugs present in the capsule formulation is given in Table-99 for ASP and ROSU.

The amount present in the capsule formulation was in good concord with the label claim and the %RSD values were found to be 0.11697 and 0.23497 for ASP and ROSU respectively. The %RSD values were found to be less than 2, which indicate 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 three consecutive days. The %RSD values of intraday and interday analysis were found to be 0.24849 and 0.09886 for ASP & 0.34935 and 0.30904 for ROSU. The results of analysis are shown in Table-100. The results show that the precision of the method is confirmed.

The developed method was validated for Ruggedness. In the present work, it was confirmed by different Analysts and different instruments. The %RSD value for Analyst 1 and Analyst 2 were found to be 0.04018 and 0.03929 for ASP & 0.08995 and 0.08286 for ROSU respectively. The %RSD value for Instrument I and Instrument II were found to 0.00033 and 0.03566 for ASP & 0.21159 and 1.17161 for ROSU. The low %RSD values indicate that the developed method is more rugged. The results are shown in Table-101.

The accuracy of the method was performed by recovery studies. To the preanalyzed formulation, a known quantity of ASP and ROSU raw material solutions were added at different levels. The absorbances of the solutions were measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 100.04-100.13% for ASP and 99.47-99.79% for ROSU. The low %RSD values for the two drugs indicate that this method is very accurate. The recovery data is shown in Table-102. The result indicates that there is no interference due to excipients present in the formulation ROZUCOR ASP-10. The method can be easily and conveniently adopted for routine quality control analysis. And also this method is accurate, simple, rapid, precise, reliable, sensitive, reproducible & economic, and it is validated as per ICH guidelines.

6.6.2. Absorbance ratio method:

The individual and overlaid spectra of ASP and ROSU were recorded and shown in Figures-76, 77 & 83. From the overlaid spectrum, the wavelengths selected were 243 nm (λ_{max} of ROSU) and 229.8 nm (iso-absorptive point).

The linearity of ASP and ROSU were constructed in the range of 7.5-37.5 μg/ml and 1-5 μg/ml and their calibration curves are shown in Figures 84 & 85. The optical characteristics such as Beer's law limit (7.5-37.5 and 1-5 μg/ml), molar extinction co-efficient, Sandell's sensitivity, correlation co-efficient, slope and intercept were calculated and shown in Tables 103 &104.

The amount present in the formulation was determined by calculating the average of six replicate analysis and its percentage purity was found to be in the range of 99.45-99.93% for ASP and 99.45-99.70% for ROSU. The amount present in the capsule formulation was in good concord with the label claim and the %RSD values were found to be 0.19383

and 0.11036 for ASP and ROSU respectively. The low %RSD values indicate that the method has good precision. The result of analysis is shown in Table-105.

Precision of the method was studied by making repeated analysis of the same sample and it was carried out three times in a day and for three days. The %RSD values of intraday and interday analysis were found to be 0.01559 and 0.01558 for ASP & 0.01699 and 0.00173 for ROSU. The results of the analysis are shown in Table-106. The results show that the precision of the method is very high.

The developed method was validated for ruggedness. In the present work it was confirmed by different Analysts and different instruments. The % RSD values for Analyst 1 and Analyst 2 were found to be 0.03120 and 0.03269 for ASP & 0.89702 and 0.92365 for ROSU. The %RSD value for Instrument I and Instrument II were found to 0.03485 and 0.03292 for ASP & 0.91146 and 0.95105 for ROSU. The low % RSD values indicate that the developed method is more rugged. The results are shown in Table-107.

The accuracy of the method was confirmed by recovery studies. The percentage recovery was found to be in the range of 99.89-100.01% for ASP and 99.51-99.85% for ROSU. The %RSD values were found to be less than 2 and thus indicate that the method is accurate. The result of recovery study is shown in Table-108.

Summary

&

Conclusion

7.0 SUMMARY AND CONCLUSION

DOXOFYLLINE AND AMBROXOL HYDROCHLORIDE

Doxofylline is chemically 7-(1, 3-Dioxolan-2-yl methyl)-3, 7-dihydro-1, 3-dimethyl-1H-Purine-2, 6-Dione. It is a novel bronchodilator. Ambroxol Hydrochloride is chemically 1 ({[2-Amino-3, 5 dibromo phenyl]-methyl} amino) cyclohexanol monohydrochloride which is a semi synthetic derivative of vasicine from the Indian shrub "Adhatoda Vasica". It is a mucolytic agent and expectorant. Ambroxol Hydrochloride is an N-desmethyl metabolite of bromohexine.

Ambroxol Hydrochloride and Doxofylline in combination are used as an antiasthmatic agent. The simple, rapid, precise and reproducible analytical methods for the simultaneous estimation of Ambroxol Hydrochloride and Doxofylline in formulation were developed.

The tablet dosage form (SYNASMA-AX) containing 400 mg of Doxofylline and 30 mg of Ambroxol Hydrochloride has been selected for the study.

The methods adopted for studies were

7.1 UV SPECTROSCOPIC METHOD:

UV spectrophotometric method for the estimation of Doxofylline and Ambroxol Hydrochloride in combined tablet dosage form by

1. Simultaneous Equation Method
2. Absorbance Correction Method and
3. Absorbance Ration Method

From the solubility data, distilled water is used as a common solvent. Doxofylline and Ambroxol Hydrochloride were prepared separately (10 μg ml^{-1}) and scanned in the UV region of 200-400 nm. From the overlaid spectra, by the observation of spectral characteristics of Doxofylline and Ambroxol Hydrochloride, they were selected for Simultaneous equation method, Absorbance correction method and Absorbance ratio method. The wavelengths selected for simultaneous equation method were 274 nm and 244.5 nm, 274 nm and 308 nm for the absorbance correction method and 233.5 nm and 244.5 nm for absorbance ratio method.

1. Simultaneous Equation Method

The percentage label claim present in tablet formulation was found to be 99.97% and 98.64% for Doxofylline and Ambroxol Hydrochloride respectively. The percentage recovery was found to be in the range of 99.98-100.09% and 98.42-99.86% for Doxofylline and Ambroxol Hydrochloride respectively.

2. Absorbance Correction Method

The percentage label claim present in tablet formulation was found to be 100.32% and 99.60% for Doxofylline and Ambroxol Hydrochloride respectively. The percentage recovery was found to be in the range of 99.64-100.00% and 99.73-100.55% for Doxofylline and Ambroxol Hydrochloride respectively.

3. Absorbance Ratio Method

The percentage label claim present in tablet formulation was found to be 99.57% and 98.88% for Doxofylline and Ambroxol Hydrochloride respectively. The percentage

recovery was found to be in the range of 100.30-100.50% and 99.00-99.83% for Doxofylline and Ambroxol Hydrochloride respectively.

7.2 REVERSE PHASE HIGH-PERFORMANCE LIQUID CHROMOTOGRAPHY METHOD:

RP-HPLC method has been developed for the estimation of both drugs in bulk and in formulation. The proposed method gives reliable assay results with short analysis time, using mobile phase Phosphate buffer, pH 3.0: Acetonitrile: Methanol in the ratio of 70: 20: 10. The percentage label claim present in tablet formulation was found to be 100.82% and 100.12% for Doxofylline and Ambroxol Hydrochloride respectively. The percentage recovery was found to be in the range of 98.54-99.43% and 98.36-99.76% for Doxofylline and Ambroxol Hydrochloride respectively. The contents of drug present in the formulation were found to be satisfactory and system suitability parameters are in desired limit.

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

METOLAZONE AND SPIRONOLACTONE:

Metolazone is chemically 7-chloro-2-methyl-4-oxo-3-o-tolyl-1,2,3,4-tetrahydroquinazoline-6-sulfonamide. It is an antihypertensive and diuretic agent. Spironolactone is chemically 7α-acetyl thio-3-oxo-17α pregn-4-ene-21, 17β-carbolactone. It is an aldosterone antagonist and employed as a diuretic drug.

Metolazone and Spironolactone in combined tablet dosage form is used as a diuretic agent.

METOLACTONE-5 containing Metolazone 5 mg and Spironolactone 50 mg has been selected for the study.

7.3 UV-SPECTROSCOPIC METHOD:

UV spectrophotometric methods for the estimation of Metolazone and Spironolactone in combined tablet dosage form by

1. Simultaneous Equation Method
2. Absorbance Correction Method and
3. Derivative Spectroscopic method

From the solubility data, methanol is used as a common solvent. Metolazone and Spironolactone were prepared separately (10 $\mu g\ ml^{-1}$) and scanned in the UV region.

From the overlaid spectrum, by the observation of spectral characteristics of Metolazone and Spironolactone, they were selected for Simultaneous estimation method, Absorbance correction method and Derivative spectroscopic method. The wavelengths selected for simultaneous estimation method were 236.5 nm and 242.5 nm, 242.5 nm and 345 nm for the absorbance correction method and 266 nm and 289 nm for first order derivative spectroscopic method.

1. Simultaneous Equation Method

The percentage label claim present in tablet formulation was found to be 100.37% and 100.45% for Metolazone and Spironolactone respectively. The percentage recovery was

found to be in the range of 99.67-100.27% and 99.41-101.36% for Metolazone and Spironolactone respectively.

2. Absorbance Correction Method

The percentage label claim present in tablet formulation was found to be 100.17% and 100.06% for Metolazone and Spironolactone respectively. The percentage recovery was found to be in the range of 99.95-100.29% and 100.52-100.63% for Metolazone and Spironolactone respectively.

3. Derivative Spectroscopic Method

The percentage label claim present in tablet formulation was found to be 100.20% and 99.97% for Metolazone and Spironolactone respectively. The percentage recovery was found to be in the range of 99.81-100.92% and 100.16-100.31% for Metolazone and Spironolactone respectively.

7.4 REVERSE PHASE HIGH-PERFORMANCE LIQUID CHROMOTOGRAPHY METHOD:

RP-HPLC method was developed for the simultaneous determination of Metolazone and Spironolactone, and are validated as per ICH guidelines. The regression coefficient (r^2) for each analyte is around 0.999 which shows good linearity. The developed method gives reliable assay results with short analysis time, using mobile phase Phosphate buffer 25 mM (pH 3.5): Acetonitrile: Methanol in the ratio of 40: 30: 30. The percentage label claim present in tablet formulation was found to be 99.23% and 100.29% for Metolazone and Spironolactone respectively. The percentage recovery was found to be in the range of 99.74-100.33% and 99.93-101.08% for Metolazone and Spironolactone respectively. The good % recovery in the tablet dosage form reveals that the excipients present in the

dosage form have no interference in the determination. The %RSD was also less than 2 showing high degree of precision.

The contents of drug present in the formulation were found to be satisfactory and system suitability parameters are in desired limit. All the above methods are suitable for the reliable analysis of commercial formulations containing combinations of Metolazone and Spironolactone. Thus the methods are simple, precise, rapid and accurate.

Since the method do not require use of expensive reagent and also less time consuming, it can be performed routinely in industry for analysis of analytes in pharmaceutical dosage forms.

METOPROLOL AND OLMESARTAN:

Metoprolol is chemically (±) 1-(isopropylamino)-3-[p-(2-methoxyethyl)-phenoxy)-2-propanol. It is an anti hypertensive agent. Olmesartan is (5-methyl-2-oxo-2H-1,3-dioxol-4-yl)methyl 4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)-1H-imidazole-5-carboxylate. It is an angiotension II receptor blocker and used as an antihypertensive agent.

Metoproprolol and Olmesartan in combined dosage form is employed as an antihypertensive agent.

The simple, rapid, precise and reproducible analytical methods for the simultaneous estimation of Metoprolol and Olmesartan in the formulations were developed.

The OLSAR-M 25 (Capsule) and OLMESAR-M (Tablet) containing 25 mg of METO and 20 mg of OLME, and OLMAX-M (Tablet) containing 50 mg of METO and 20 mg of OLME, have been selected for the study.

The methods adopted for studies were

7.5 UV-SPECTROSCOPIC METHOD

UV spectrophotometric methods for the estimation of Metoprolol and Olmesartan in combined capsule/tablet dosage form are

1. Simultaneous Equation Method

2. Area Under the Curve Method and

3. Derivative Spectroscopic Method

From the solubility data, methanol is used as a common solvent. Metoprolol and Olmesartan were prepared separately (10 μg ml^{-1}) and scanned in the UV region. From the overlaid spectra, by the observation of spectral characteristics of Metoprolol and Olmesartan, they were selected for Simultaneous Equation method, Area Under the Curve method and Derivative Spectroscopic method. The wavelengths selected for simultaneous equation method were 223.5 nm and 256.5 nm, 218-228 nm and 246-266 nm for area under the curve method and 256 nm and 243 nm for derivative spectroscopic method.

7.5.1. Simultaneous Equation Method

OLSAR-M 25: The percentage label claim present in capsule formulation was found to be 100.09% and 99.30% for Metoprolol and Olmesartan respectively. The percentage recovery was found to be in the range of 99.98-100.48% and 100.29-101.08% for Metoprolol and Olmesartan respectively.

OLMESAR-M: The percentage label claim present in tablet formulation was found to be 100.20% and 99.33% for Metoprolol and Olmesartan respectively. The percentage recovery was found to be in the range of 99.59-100.51% and 100.34-101.23% for Metoprolol and Olmesartan respectively.

OLMAX-M: The percentage label claim present in tablet formulation was found to be 100.28% and 99.60% for Metoprolol and Olmesartan respectively. The percentage recovery was found to be in the range of 100.07-100.16% and 99.70-101.30% for Metoprolol and Olmesartan respectively.

7.5.2. Area Under the Curve Method

OLSAR-M 25: The percentage label claim present in capsule formulation was found to be 100.07% and 99.87% for Metoprolol and Olmesartan respectively. The percentage recovery was found to be in the range of 99.78-100.92% and 99.89-100.17% for Metoprolol and Olmesartan respectively

OLMESAR-M: The percentage label claim present in tablet formulation was found to be 100.09% and 99.77% for Metoprolol and Olmesartan respectively. The percentage recovery was found to be in the range of 99.32-100.37% and 98.75-100.32% for Metoprolol and Olmesartan respectively

OLMAX-M: The percentage label claim present in tablet formulation was found to be 100.11% and 99.83% for Metoprolol and Olmesartan respectively. The percentage recovery was found to be in the range of 100.12-100.15% and 100.05-100.20% for Metoprolol and Olmesartan respectively

7.5.3. Derivative Spectroscopic Method

OLSAR-M 25: The percentage label claim present in capsule formulation was found to be 99.96% and 100.06% for Metoprolol and Olmesartan respectively. The percentage recovery was found to be in the range of 100.21-100.47% and for both Metoprolol and Olmesartan.

OLMESAR-M: The percentage label claim present in tablet formulation was found to be 99.81% and 100.00% for Metoprolol and Olmesartan respectively. The percentage recovery was found to be in the range of 100.64-100.76% and 99.73-99.92% for Metoprolol and Olmesartan respectively

OLMAX-M: The percentage label claim present in tablet formulation was found to be 100.51% and 100.15% for Metoprolol and Olmesartan respectively. The percentage recovery was found to be in the range of 100.03-100.05% and 99.95-100.25% for Metoprolol and Olmesartan respectively

All the above methods are simple, precise, economic, rapid and accurate and the developed methods are suitable for determination of Metoprolol and Olmesartan as bulk drug and in marketed dosage form without any interference from the excipients.

Statistical analysis proves that these methods are repeatable and selective for the analysis of Metoprolol and Olmesartan.

ASPIRIN AND ROSUVASTATIN

Aspirin is chemically 2-acetoxybenzoic acid or acetylsalicylic acid, which is best known as an anti-platelet drug. It is also used as analgesic, antipyretic and anti-inflammatory drug. Rosuvastatin is chemically (3R,5S,6E)-7-[4-(4-flurophenyly)-2-(N-

methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5 dihydroxyhept-6-enoic acid. Rosuvastatin is a member of the class of statins, used to treat hypercholesterolemia and related conditions and to prevent cardiovascular disease.

Aspirin and Rosuvastatin in combination is used in cardiovascular diseases.

The simple, rapid, precise and reproducible analytical methods for the simultaneous estimation of Aspirin and Rosuvastatin in formulation were developed.

The capsule dosage form (**ROZUCOR ASP-10**) containing 75 mg of Aspirin and 10 mg of Rosuvastatin has been selected for the study.

The methods adopted for studies were

7.6 UV-SPECTROSCOPIC METHODS:

UV spectrophotometric methods for the estimation of Aspirin and Rosuvastatin in combined capsule dosage form by

1. Simultaneous Equation Method and

2. Absorbance Ratio Method

From the solubility data, methanol is used as a common solvent. Aspirin and Rosuvastatin were prepared separately (10 $\mu g\ ml^{-1}$) and scanned in the UV region of 200-400 nm. From the overlaid spectrum, by the observation of spectral characteristics of Aspirin and Rosuvastatin, they were selected for Simultaneous equation method and Absorbance ratio method. The wavelengths selected for simultaneous equation method were 294.5 nm & 243 nm, and 229.8 nm & 243 nm for the absorbance ratio method.

1. Simultaneous Equation Method

The percentage label claim present in the capsule formulation was found to be 99.81% and 99.46% for Aspirin and Rosuvastatin respectively. The percentage recovery was found to be in the range of 100.04-100.13% and 99.47-99.79% for Aspirin and Rosuvastatin respectively.

2. Absorbance Ratio Method

The percentage label claim present in capsule formulation was found to be 99.77% and 99.59% for Aspirin and Rosuvastatin respectively. The percentage recovery was found to be in the range of 99.89-100.01% and 99.51-99.85% for Aspirin and Rosuvastatin respectively.

The two methods are simple, precise, economic, rapid and accurate and the developed methods are suitable for determination of Aspirin and Rosuvastatin as a bulk drug and in marketed dosage form without any interference from the excipients. Statistical analysis proves that these methods are repeatable and selective for the analysis of Aspirin and Rosuvastatin.

Impact of the study

8. IMPACT OF THE STUDY

The proposed analytical methods are simple, accurate and reproducible. The advantages lie in the simplicity of sample preparation and the cost economic reagents used.

The contribution is the limit of detection for all the methods. Results from statistical analysis of the experimental results for all the methods were indicative of satisfactory precision and reproducibility. Hence the spectrophotometric methods and HPLC method can be used for analysis of the different solid dosage formulations in commercial quality control laboratories. All the above methods do not suffer from any interference due to common excipients. Therefore it was shown that the proposed methods could be successfully applied to estimate the commercial pharmaceutical products containing Doxofylline & Ambroxol Hydrochloride, Metolazone & Spironolactone, Metoprolol & Olmesartan and Aspirin & Rosuvastatin. Thus the above studies findings would be helpful to the analytical chemists to apply the analytical methods for the routine analysis of the analytes in pharmaceutical dosage forms after their approval from FDA. However the following aspects of the method may also be tried for future analysis

1. HPTLC for formulation.
2. Gas Chromotragraphic analysis
3. Liquid Chromatography Coupled to Tandem Mass Spectrometry
4. High Performance Liquid Chromatography with Fluorescence Detection
5. Colorimetric method development
6. In the presence of another drug

This study also paves the platform for the estimation of analytes in biological fluids.

Appendix-
List
of
Figures

FIGURE-1
UV SPECTRUM OF DOXOFYLLINE
IN DISTILLED WATER
CONCENTRATION: 10 μg ml^{-1}

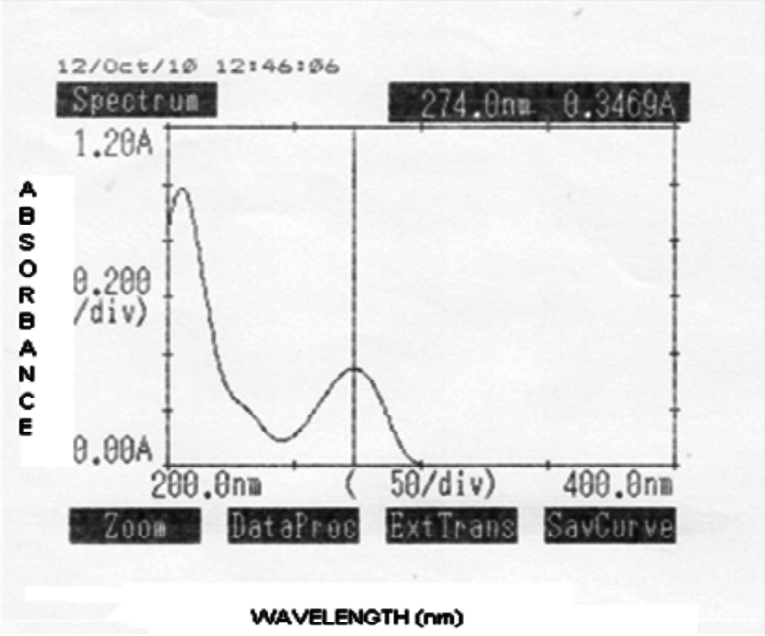


FIGURE-2
UV SPECTRUM OF AMBROXOL HYDROCHLORIDE
IN DISTILLED WATER
CONCENTRATION: 10 μg ml^{-1}

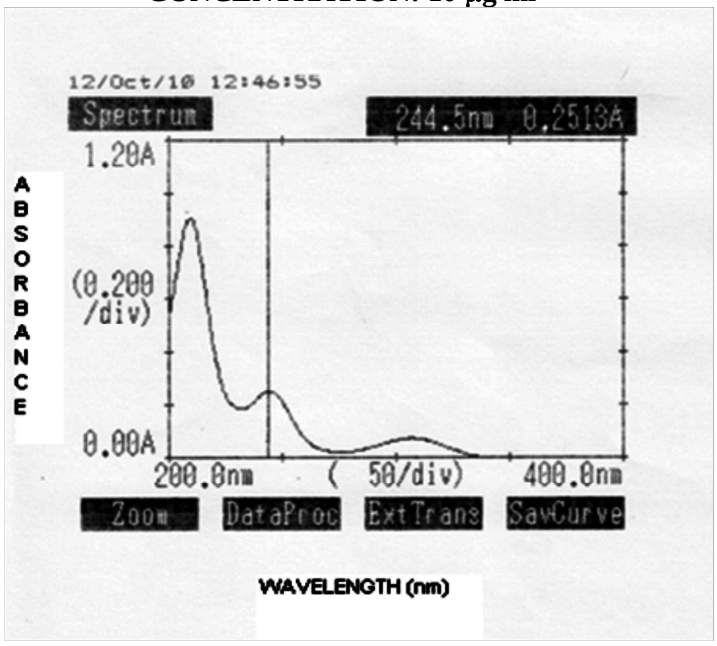


FIGURE-3
OVERLAID SPECTRUM OF DOXOFYLLINE AND AMBROXOL HYDROCHORIDE IN DISTILLED WATER

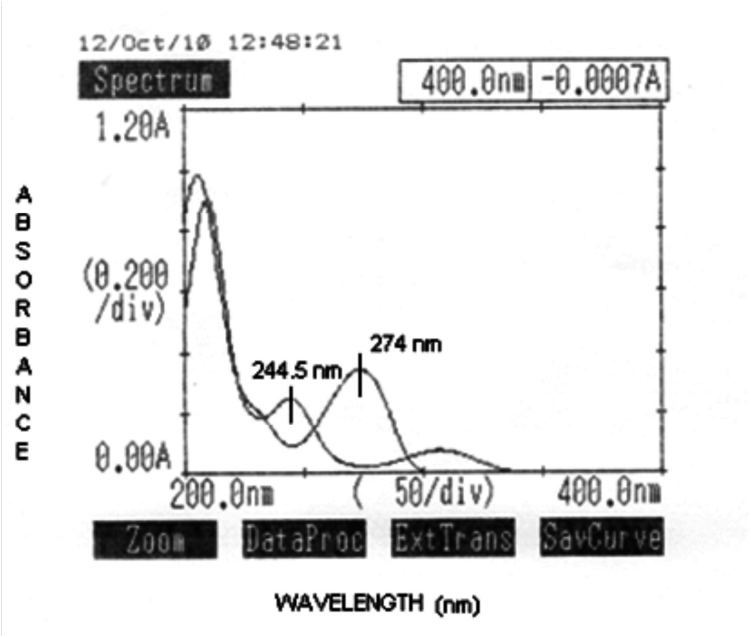


FIGURE-4
CALIBRATION CURVE OF DOXOFYLLINE IN DISTILLED WATER AT 244.5 nm

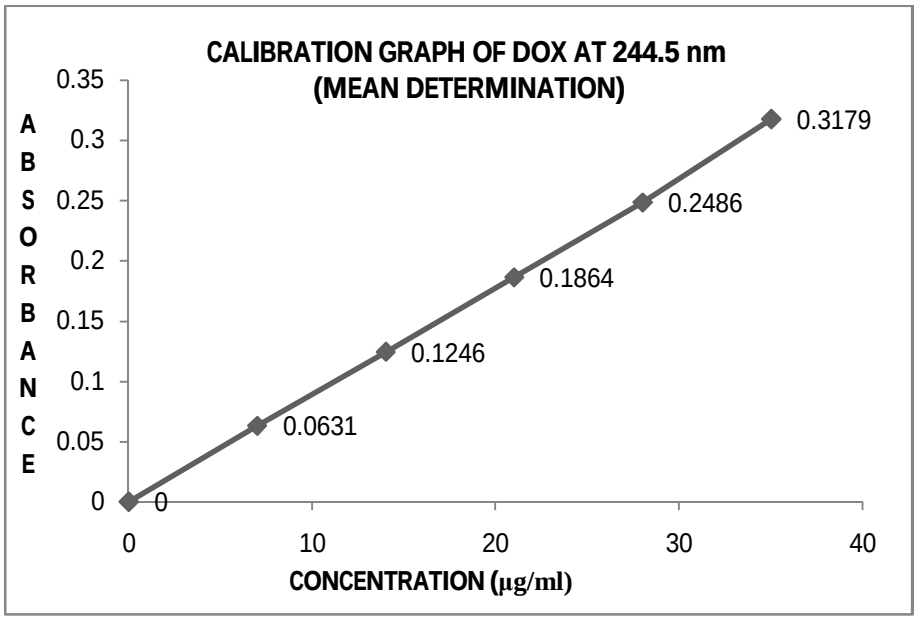


FIGURE-5
CALIBRATION CURVE OF DOXOFYLLINE
IN DISTILLED WATER AT 274 nm

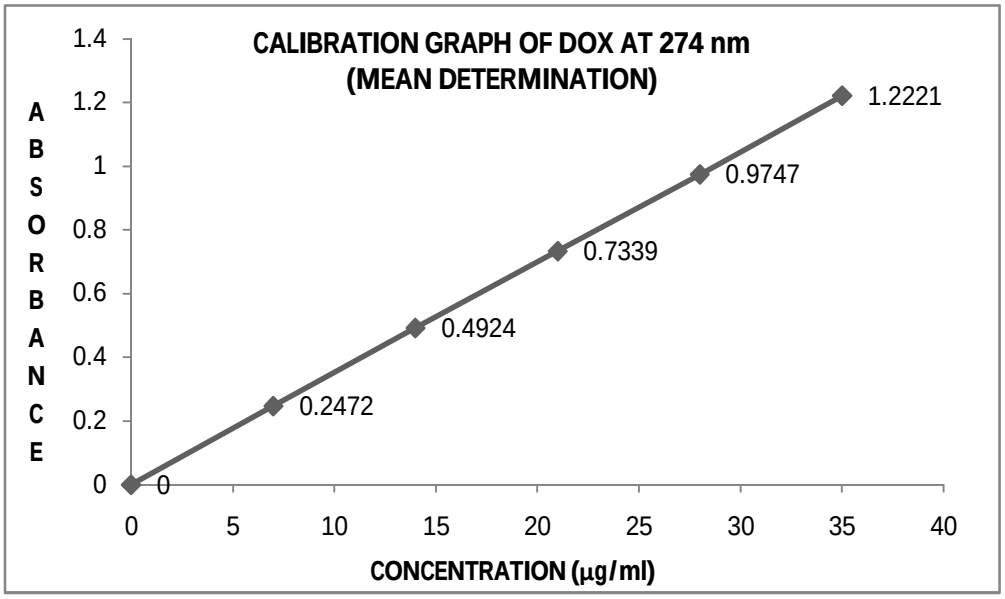


FIGURE-6
CALIBRATION CURVE OF AMBROXOL HYDROCHLORIDE
IN DISTILLED WATER AT 244.5 nm

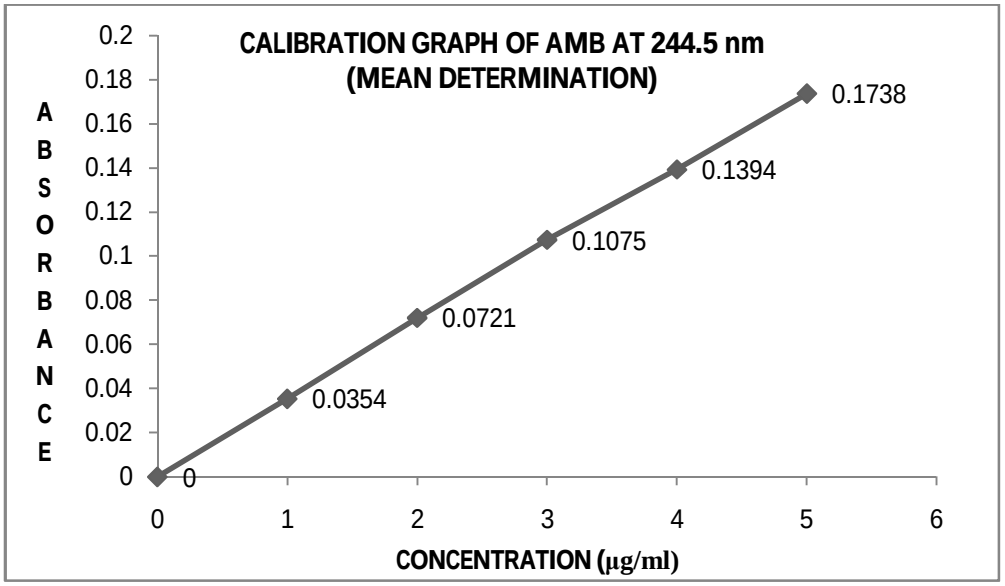


FIGURE-7
CALIBRATION CURVE OF AMBROXOL HYDROCHLORIDE IN DISTILLED WATER AT 274 nm

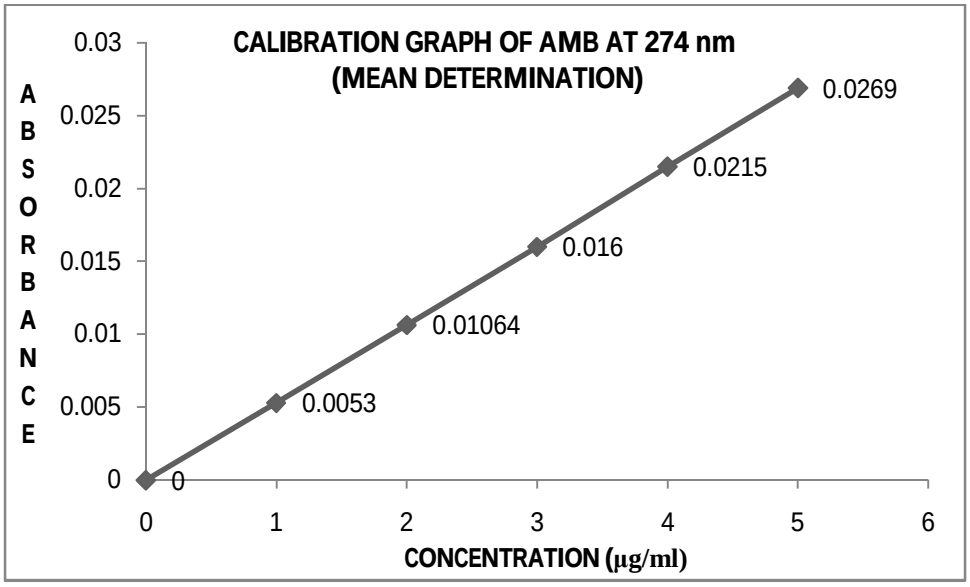


FIGURE-8
OVERLAID SPECTRUM OF DOXOFYLLINE AND AMBROXOL HYDROCHORIDE (ABSORBANCE CORRECTION METHOD)

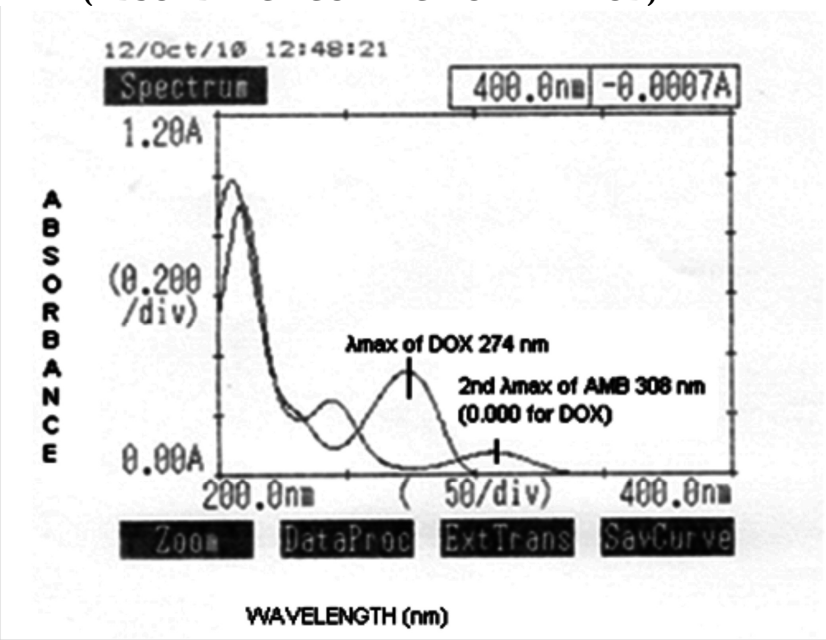


FIGURE-9
CALIBRATION CURVE OF AMBROXOL HYDROCHLORIDE IN DISTILLED WATER AT 308 nm

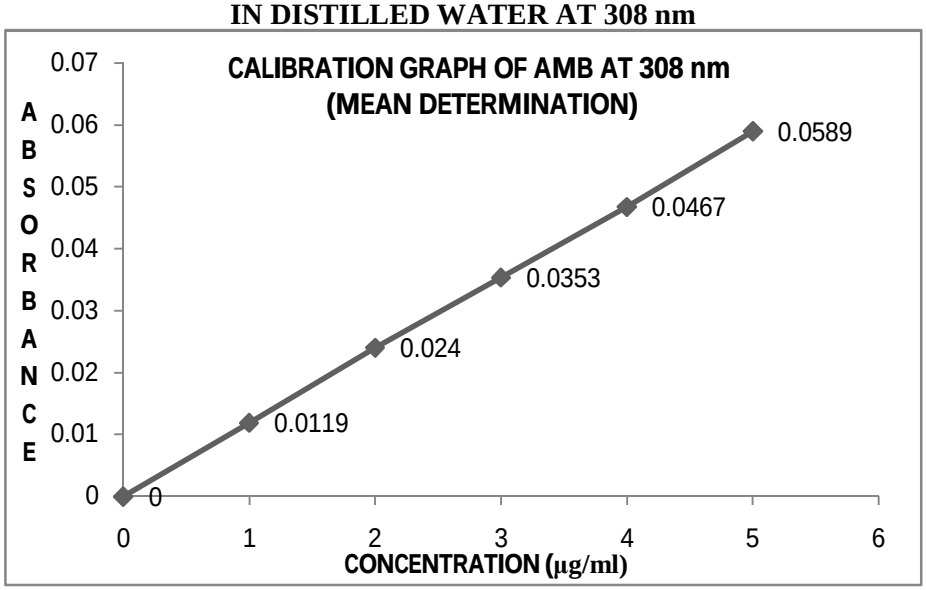


FIGURE-10
OVERLAID SPECTRUM OF DOXOFYLLINE AND AMBROXOL HYDROCHORIDE (ABSORBANCE RATIO METHOD)

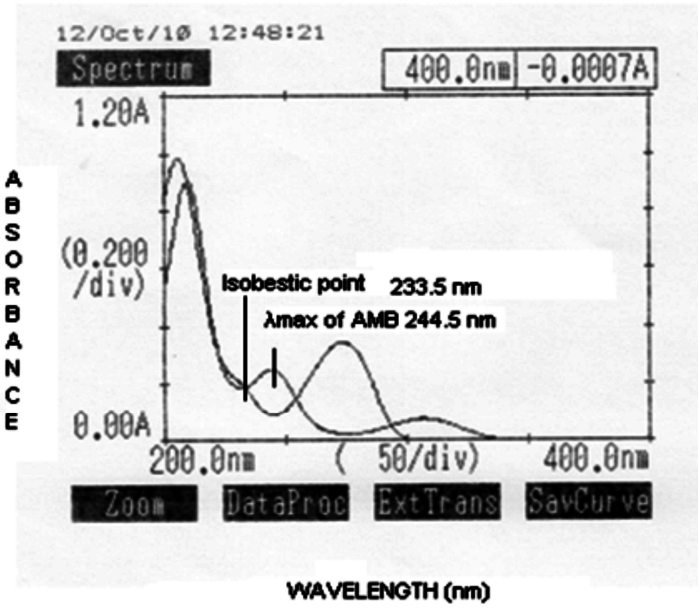


FIGURE-11
CALIBRATION CURVE OF DOXOFYLLINE
IN DISTILLED WATER AT 233.5 nm

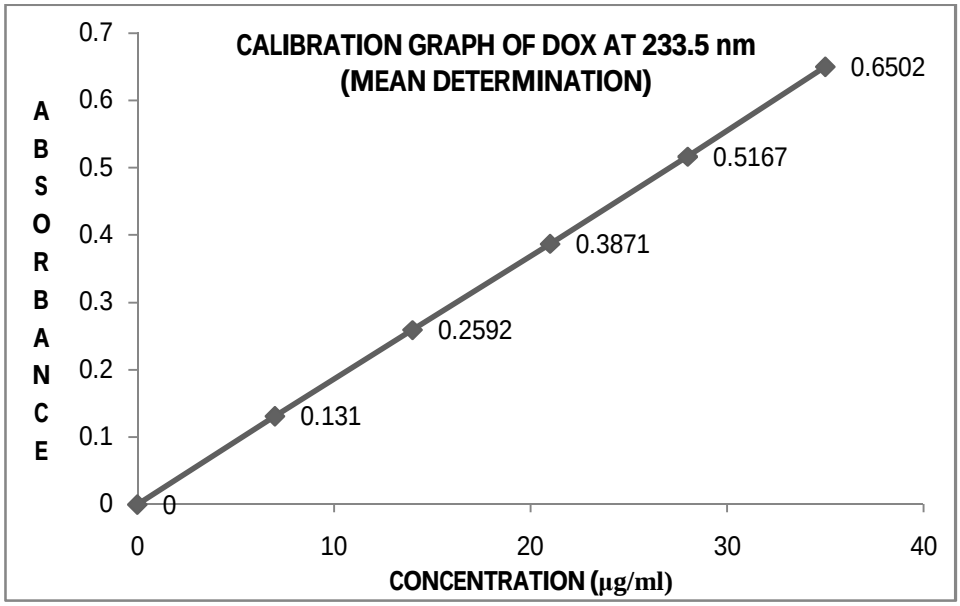


FIGURE-12
CALIBRATION CURVE OF AMBROXOL HYDROCHLORIDE
IN DISTILLED WATER AT 233.5 nm

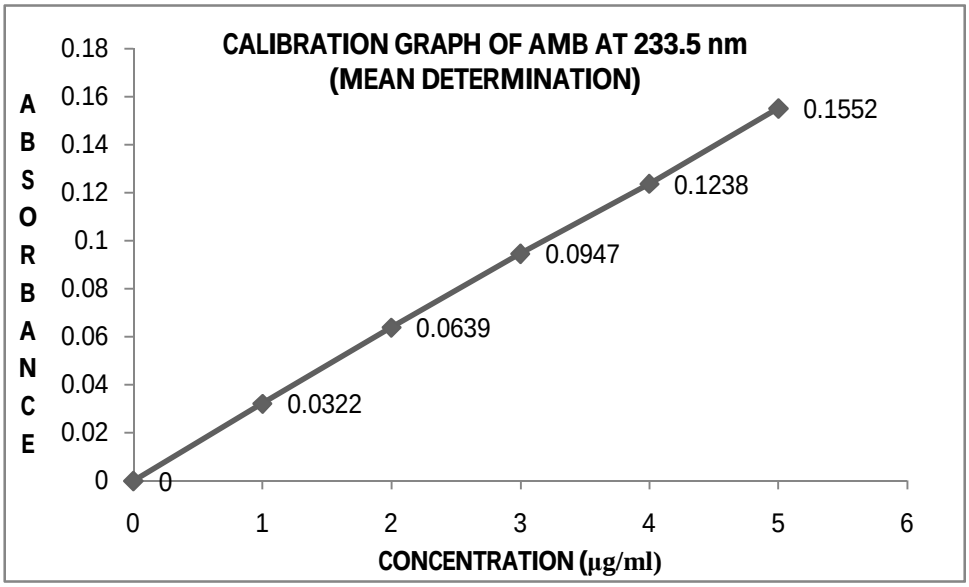
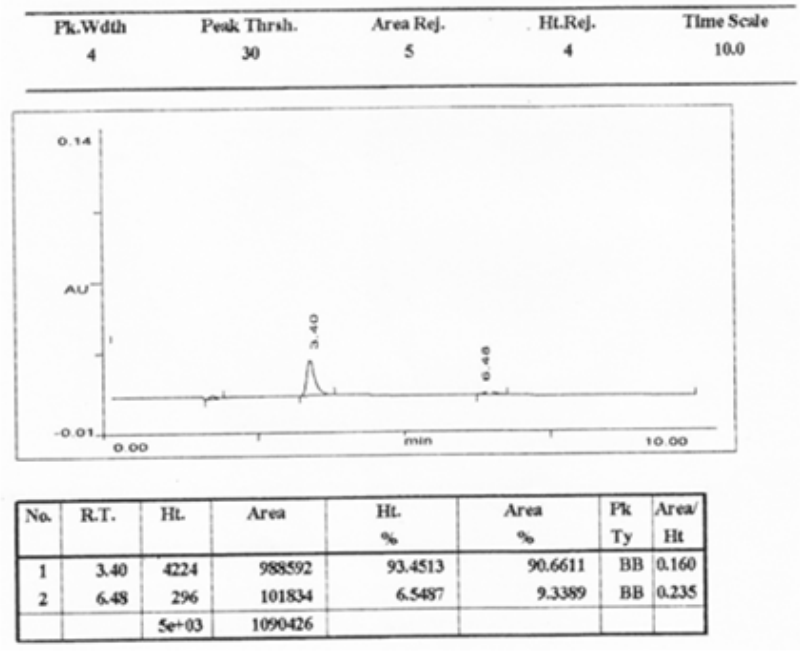
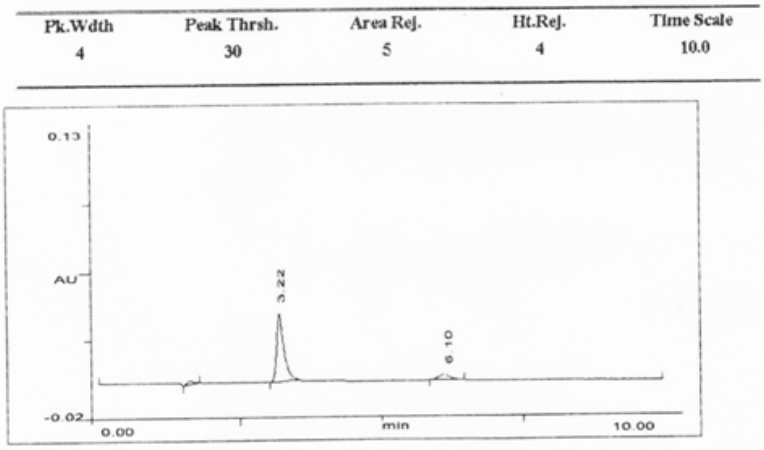


FIGURE-13
LINEARITY CHROMATOGRAM OF DOXOFYLLINE AND AMBROXOL HYDROCHLORIDE (7, 0.5 µg ml^{-1}) – FIRST SET [1/3]



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	3.40	4224	988592	93.4513	90.6611	BB	0.160
2	6.48	296	101834	6.5487	9.3389	BB	0.235
		5e+03	1090426				

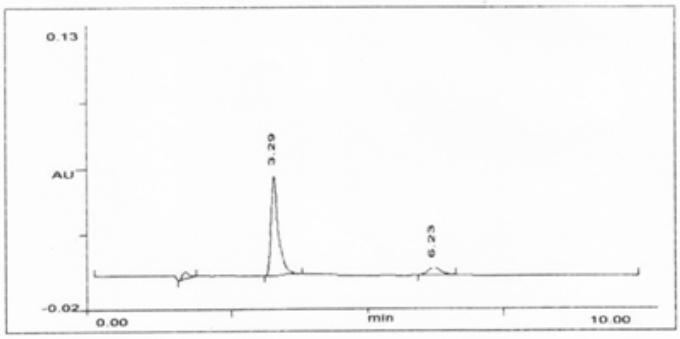
FIGURE-14
LINEARITY CHROMATOGRAM OF DOXOFYLLINE AND AMBROXOL HYDROCHLORIDE (14, 1 µg ml^{-1}) - FIRST SET [1/3]



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	3.22	8307	1896177	93.0756	89.4430	BB	0.156
2	6.10	618	223806	6.9244	10.5570	BB	0.247
		9e+03	2119983				

FIGURE-15
LINEARITY CHROMATOGRAM OF DOXOFYLLINE AND AMBROXOL HYDROCHLORIDE (21, 1.5 µg ml^{-1}) - FIRST SET [1/3]

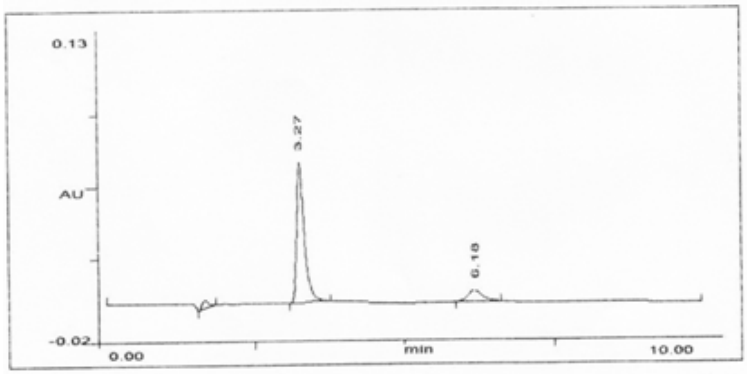
Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	10.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	3.29	12589	3134828	92.8529	89.4520	BB	0.170
2	6.23	969	369653	7.1471	10.5480	BB	0.260
		1e+04	3504481				

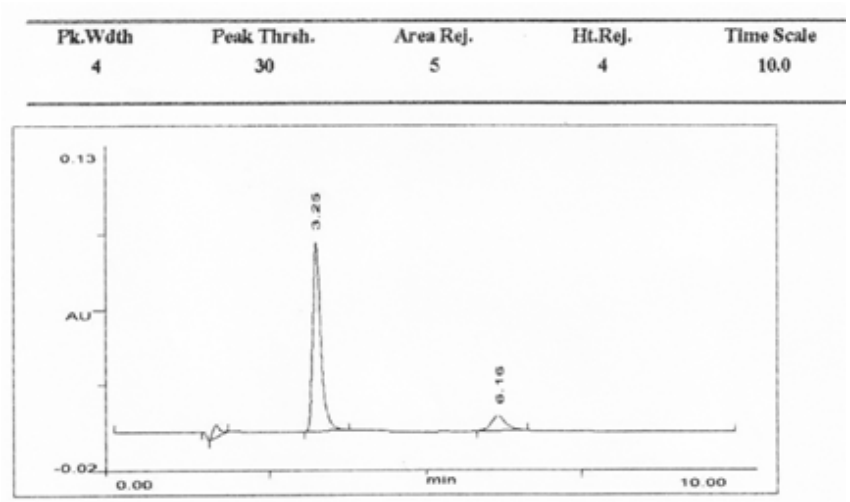
FIGURE-16
LINEARITY CHROMATOGRAM OF DOXOFYLLINE AND AMBROXOL HYDROCHLORIDE (28, 2 µg ml^{-1}) - FIRST SET [1/3]

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	10.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	3.27	16384	4098340	92.7589	89.1662	BB	0.171
2	6.18	1279	497954	7.2411	10.8338	BB	0.266
		2e+04	4596294				

FIGURE-17
LINEARITY CHROMATOGRAM OF DOXOFYLLINE AND AMBROXOL HYDROCHLORIDE (35, 2.5 µg ml^{-1}) - FIRST SET [1/3]



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	3.25	20943	5181525	92.6927	88.7385	BB	0.169
2	6.16	1651	657567	7.3072	11.2615	BB	0.272
		2e+04	5839092				

FIGURE-18
CALIBRATION CURVE OF DOXOPHYLLINE BY RP-HPLC

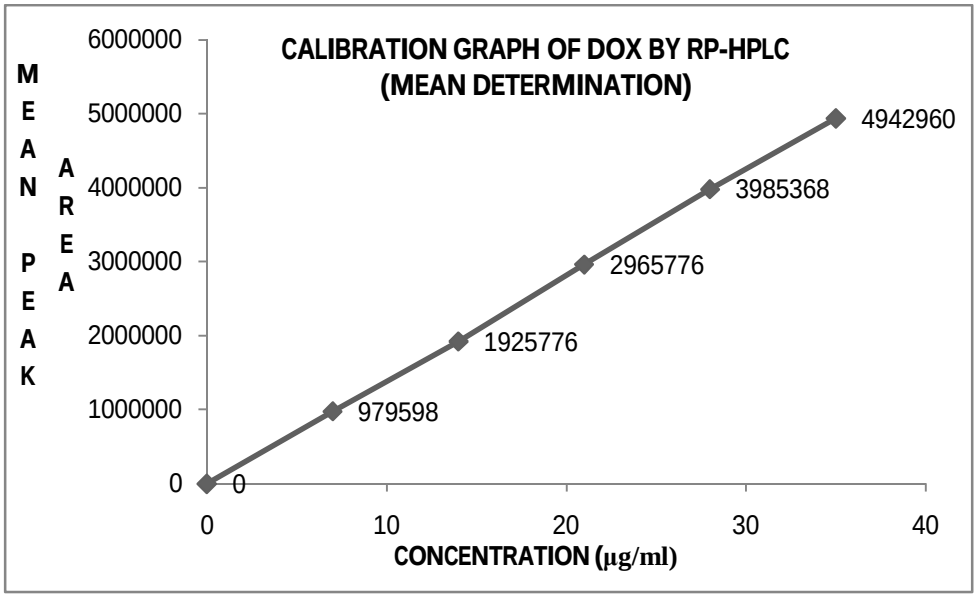


FIGURE-19
CALIBRATION CURVE OF AMBROXOL HYDROCHLORIDE BY RP-HPLC

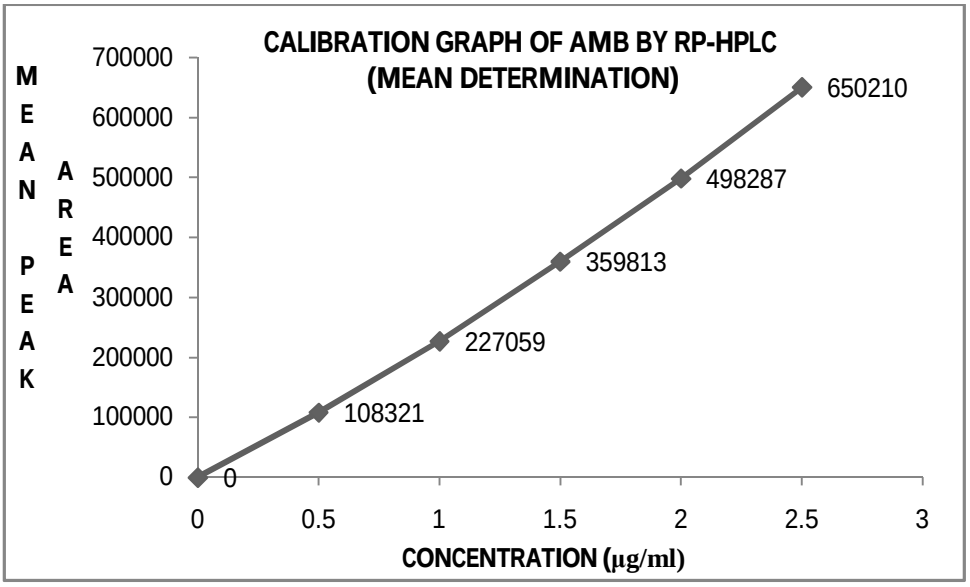
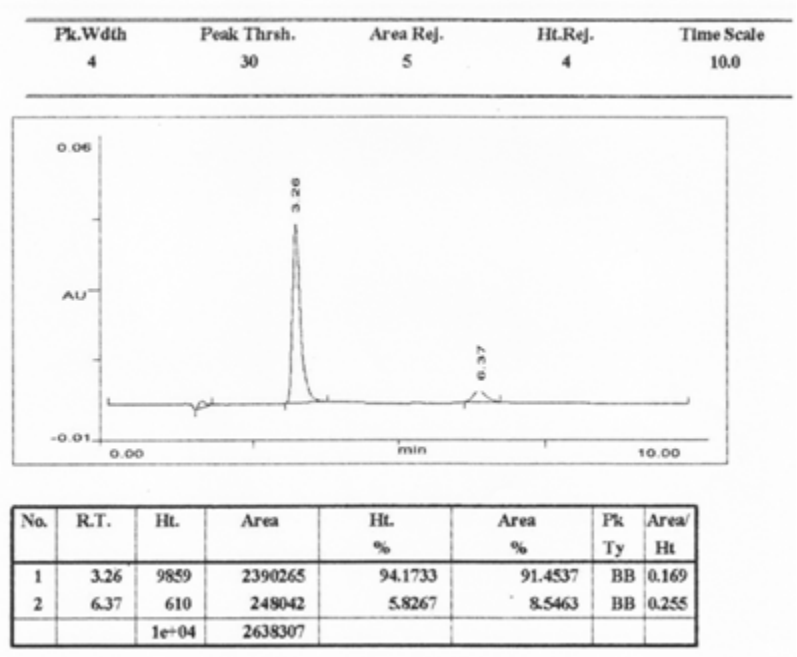
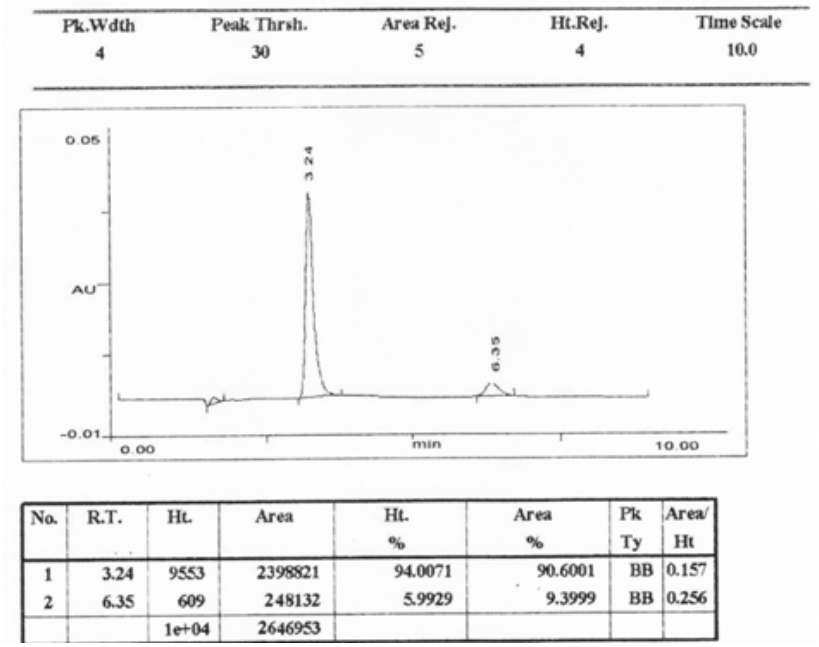


FIGURE-20
CHROMATOGRAM FOR ANALYSIS OF FOMULATION [SYNASMA-AX] REPEATABILITY -1



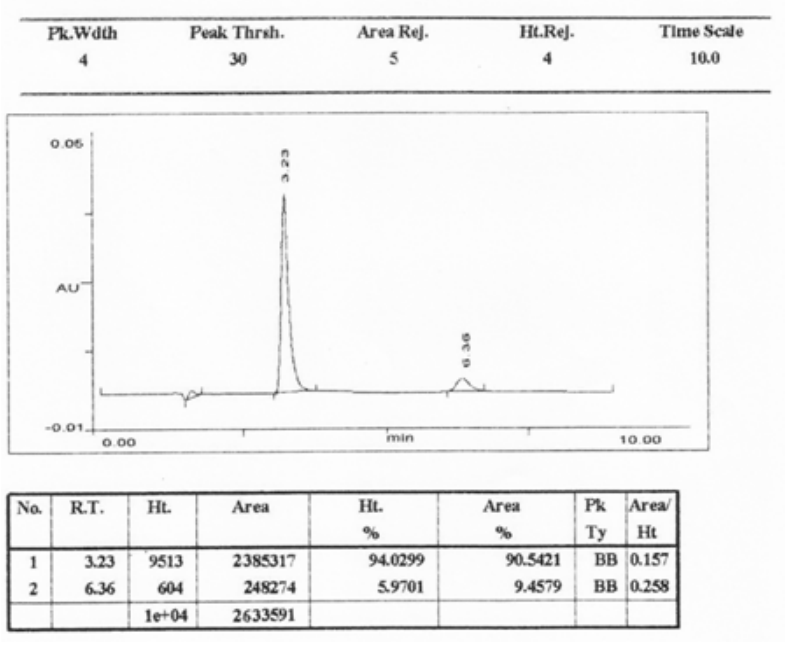
No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	3.26	9859	2390265	94.1733	91.4537	BB	0.169
2	6.37	610	248042	5.8267	8.5463	BB	0.255
		1e+04	2638307				

FIGURE-21
CHROMATOGRAM FOR ANALYSIS OF FORMULATION [SYNASMA-AX]
REPEATABILITY-2



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	3.24	9553	2398821	94.0071	90.6001	BB	0.157
2	6.35	609	248132	5.9929	9.3999	BB	0.256
		1e+04	2646953				

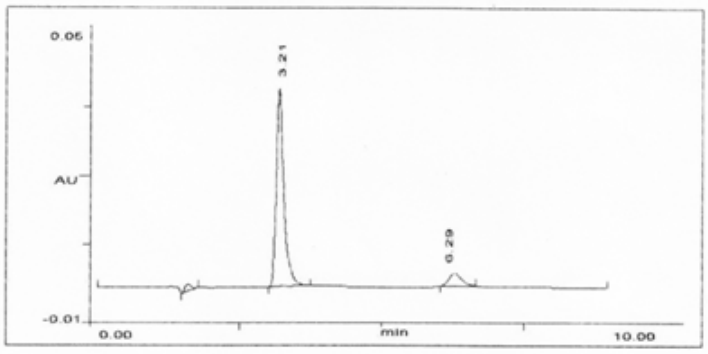
FIGURE-22
CHROMATOGRAM FOR ANALYSIS OF FORMULATION [SYNASMA-AX]
REPEATABILITY-3



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	3.23	9513	2385317	94.0299	90.5421	BB	0.157
2	6.36	604	248274	5.9701	9.4579	BB	0.258
		1e+04	2633591				

FIGURE-23
CHROMATOGRAM FOR ANALYSIS OF FORMULATION [SYNASMA-AX]
REPEATABILITY-4

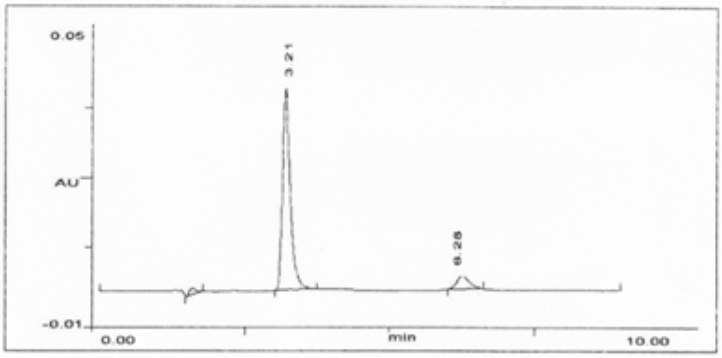
Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	10.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	3.21	9528	2368444	94.0573	90.6620	BB	0.155
2	6.29	602	243344	5.9427	9.3380	BB	0.253
		1e+04	2611788				

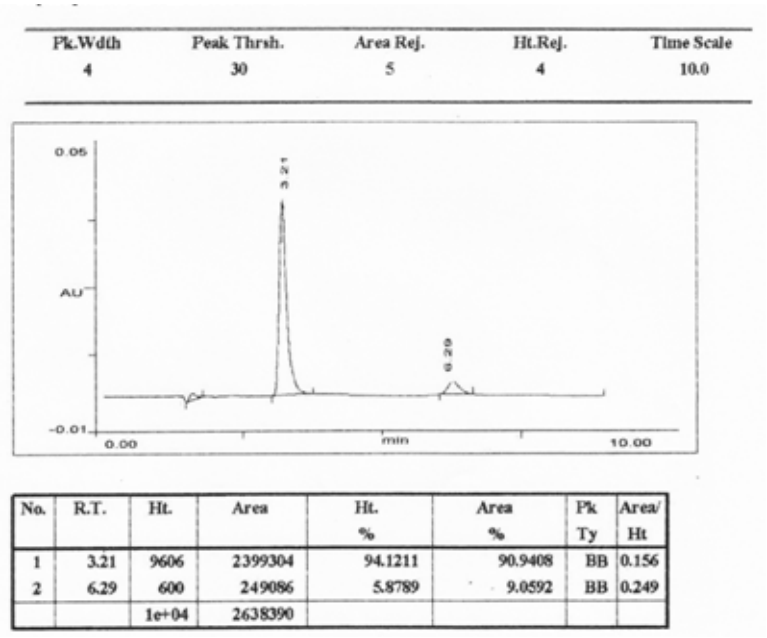
FIGURE-24
CHROMATOGRAM FOR ANALYSIS OF FORMULATION [SYNASMA-AX]
REPEATABILITY-5

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	10.0



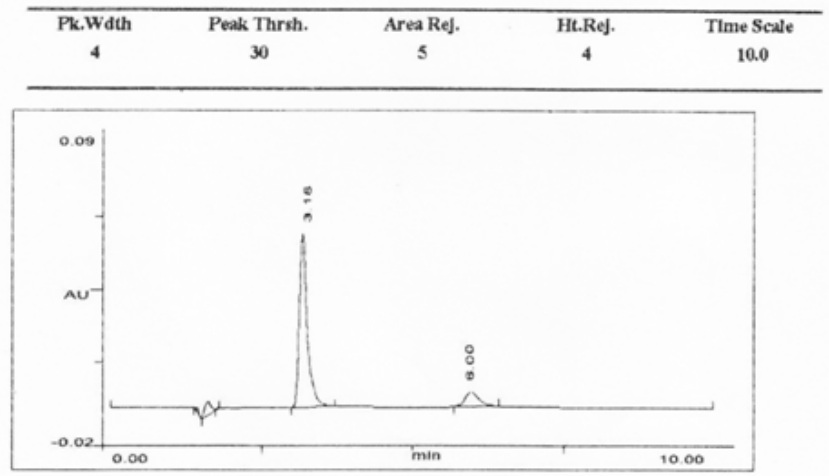
No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	3.21	9574	2392189	94.0564	90.6726	BB	0.156
2	6.28	605	245508	5.9436	9.3274	BB	0.254
		1e+04	2637697				

FIGURE-25
CHROMATOGRAM FOR ANALYSIS OF FORMULATION [SYNASMA-AX] REPEATABILITY-6



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	3.21	9606	2399304	94.1211	90.9408	BB	0.156
2	6.29	600	249086	5.8789	9.0592	BB	0.249
		1e+04	2638390				

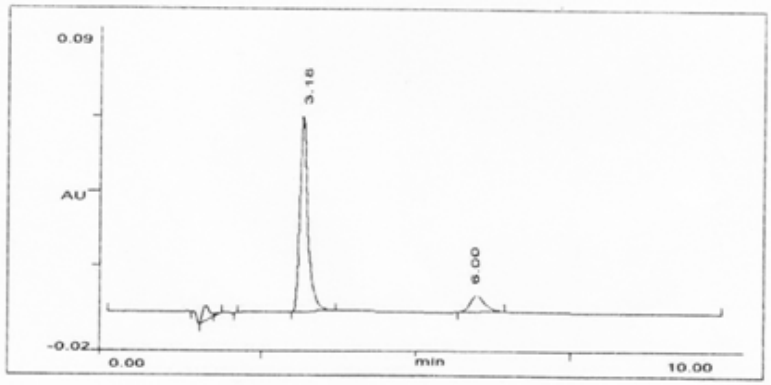
FIGURE-26
CHROMATOGRAM FOR 80% RECOVERY OF FORMULATION [SYNASMA-AX]



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	3.18	14573	4270767	92.2400	88.1678	BB	0.163
2	6.00	1226	459962	7.7600	11.8322	BB	0.259
		2e+04	4726729				

FIGURE-27
CHROMATOGRAM FOR 100% RECOVERY OF FORMULATION [SYNASMA-AX]

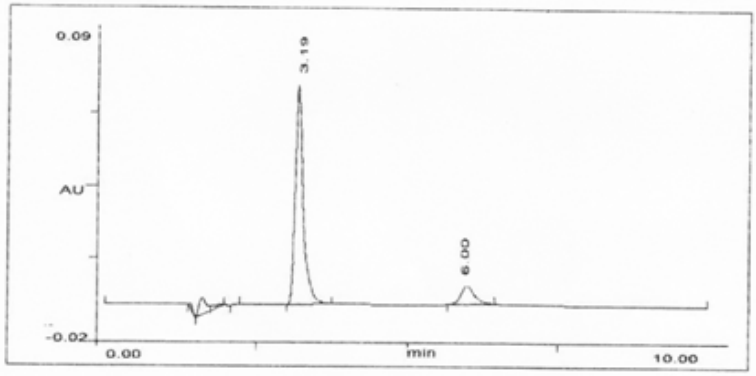
Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	10.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	3.18	16025	4742693	92.1719	87.9830	BB	0.162
2	6.00	1361	513060	7.8281	12.0170	BB	0.261
		2e+04	5255753				

FIGURE-28
CHROMATOGRAM FOR 120% RECOVERY OF FORMULATION [SYNASMA-AX]

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	10.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	3.19	18237	5226995	92.1852	88.1255	BB	0.167
2	6.00	1546	570561	7.8148	11.8745	BB	0.265
		2e+04	5797556				

FIGURE-29
UV SPECTRUM OF METOLAZONE
IN METHANOL
CONCENTRATION: 10 µg/ml

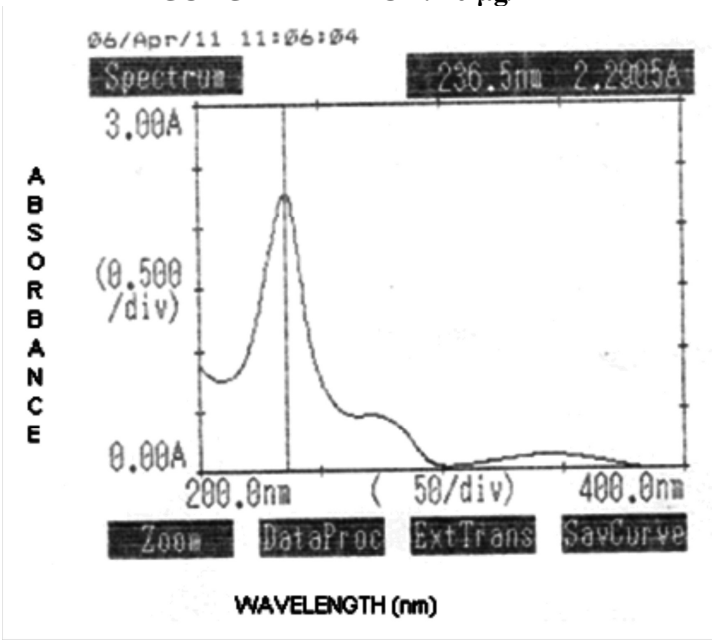


FIGURE-30
UV SPECTRUM OF SPIRONOLACTONE
IN METHANOL
CONCENTRATION: 10 µg/ml

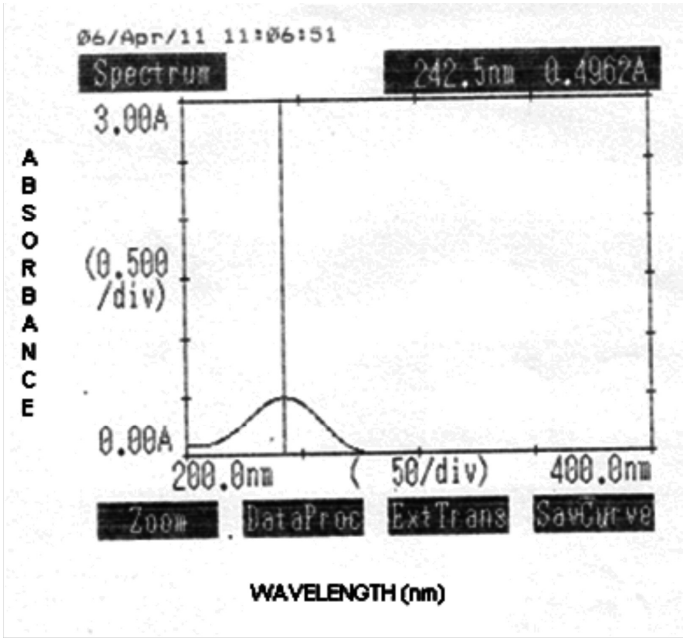


FIGURE-31
OVERLAID SPECTRA OF METOLAZONE AND SPIRONOLACTONE IN METHANOL

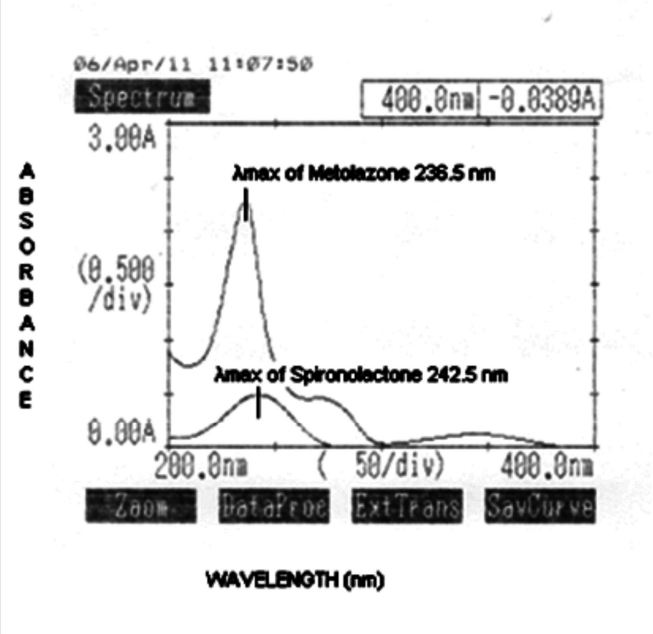


FIGURE-32
CALIBRATION CURVE OF METOLAZONE IN METHANOL AT 236.5 nm

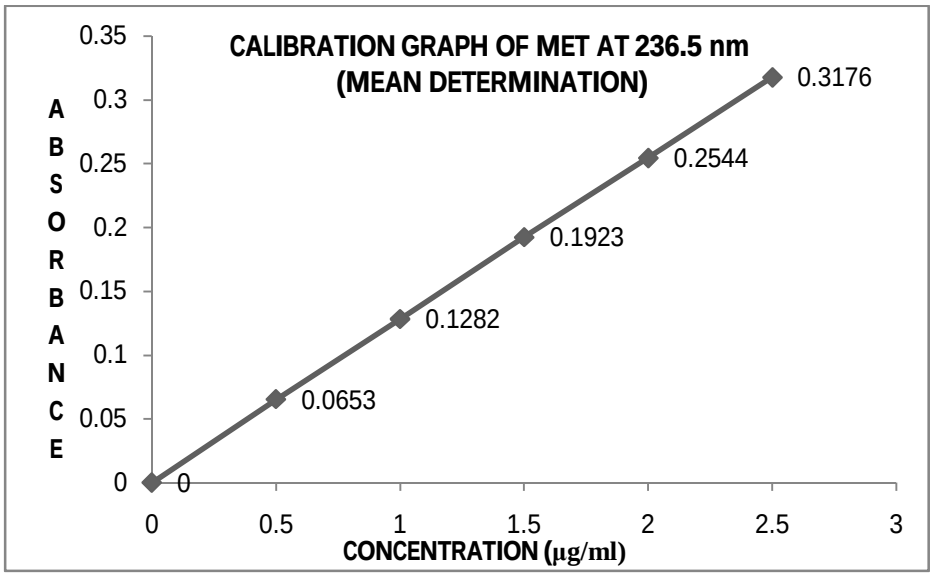


FIGURE-33
CALIBRATION CURVE OF METOLAZONE
IN METHANOL AT 242.5 nm

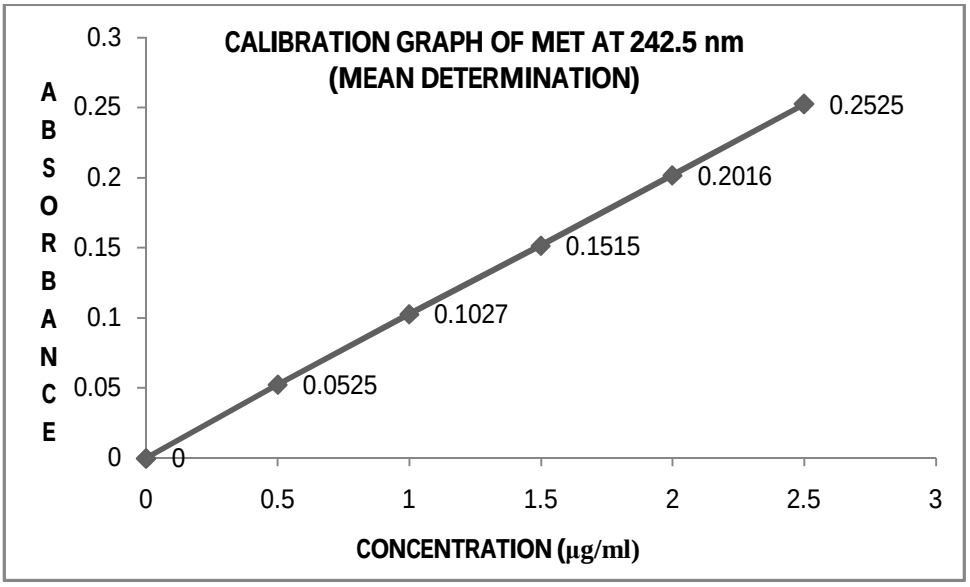


FIGURE-34
CALIBRATION CURVE OF SPIRONOLACTONE
IN METHANOL AT 236.5 nm

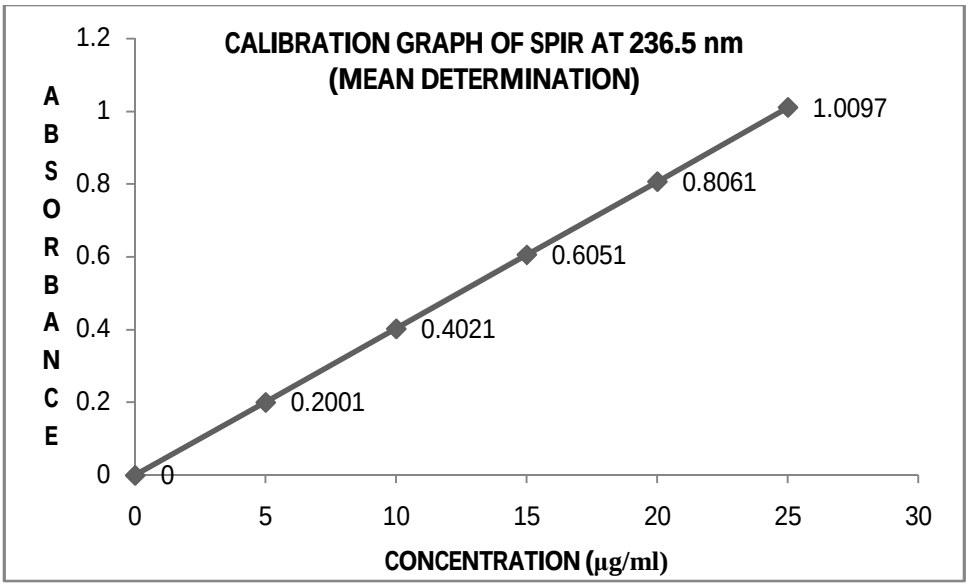


FIGURE-35
CALIBRATION CURVE OF SPIRONOLACTONE
IN METHANOL AT 242.5 nm

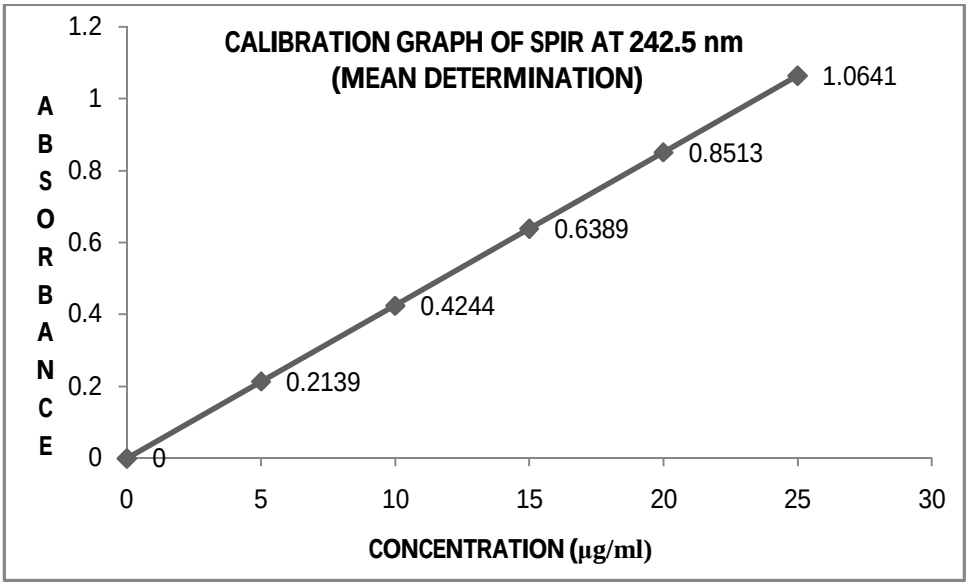


FIGURE-36
OVERLAID SPECTRUM OF METOLAZONE AND SPIRONOLACTONE
(ABSORBANCE CORRECTION METHOD)

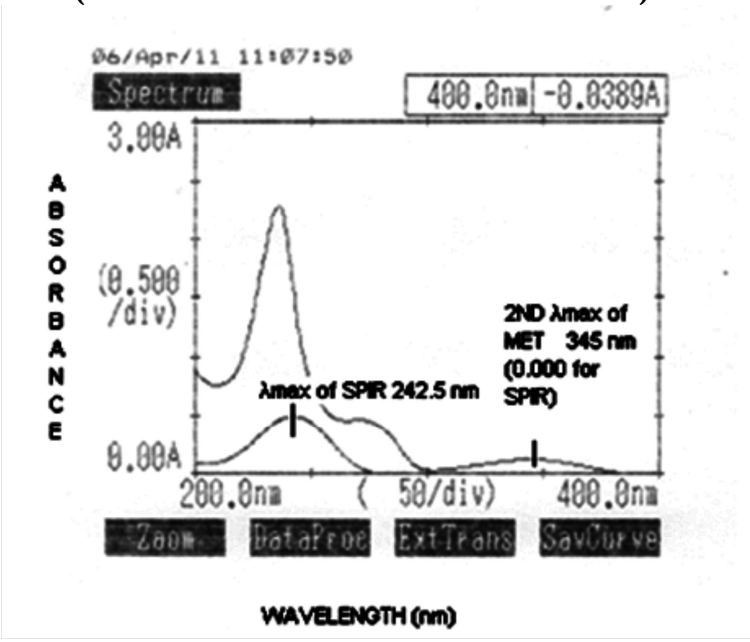


FIGURE-37
CALIBRATION CURVE OF METOLAZONE
IN METHANOL AT 345 nm

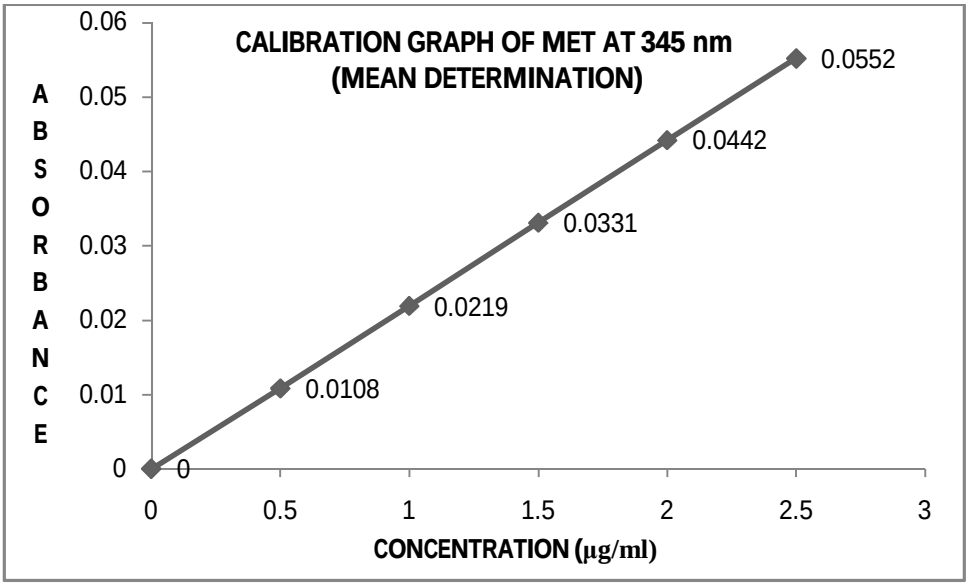


FIGURE-38
FIRST ORDER DERIVATIVE UV SPECTRUM OF METOLAZONE
IN METHANOL
(DERIVATIVE SPECTROSCOPIC METHOD)

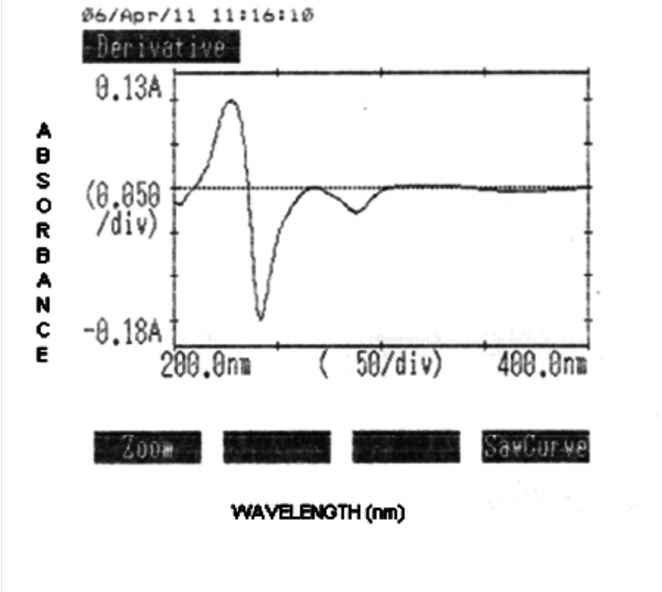


FIGURE-39
FIRST ORDER DERIVATIVE UV SPECTRUM OF SPIRONOLACTONE IN METHANOL
(DERIVATIVE SPECTROSCOPIC METHOD)

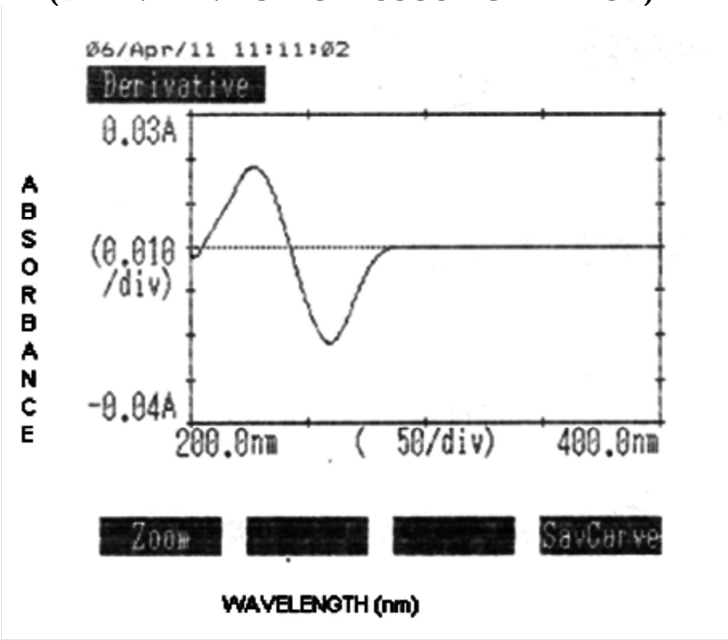


FIGURE-40
OVERLAID FIRST ORDER DERIVATIVE SPECTRUM OF METOLAZONE AND SPIRONOLACTONE IN METHANOL
(DERIVATIVE SPECTRSCOPIC METHOD)

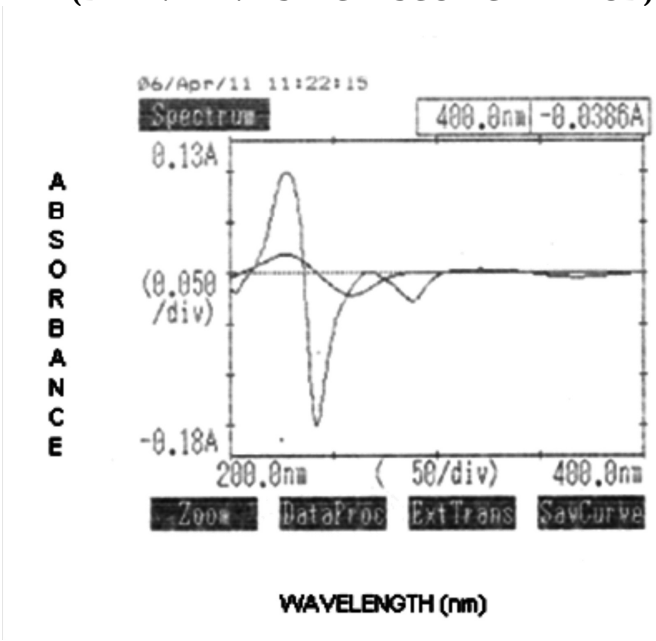


FIGURE-41
CALIBRATION CURVE OF METOLAZONE AT 289 nm
(DERIVATIVE SPECTROSCOPIC METHOD)

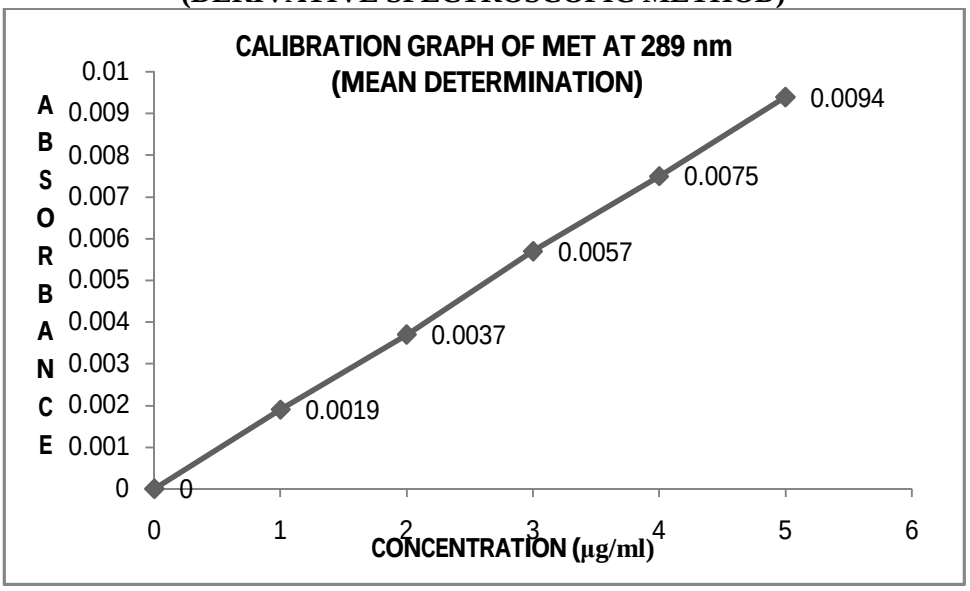


FIGURE-42
CALIBRATION CURVE OF SPIRONOLACTONE AT 266 nm
(DERIVATIVE SPECTROSCOPIC METHOD)

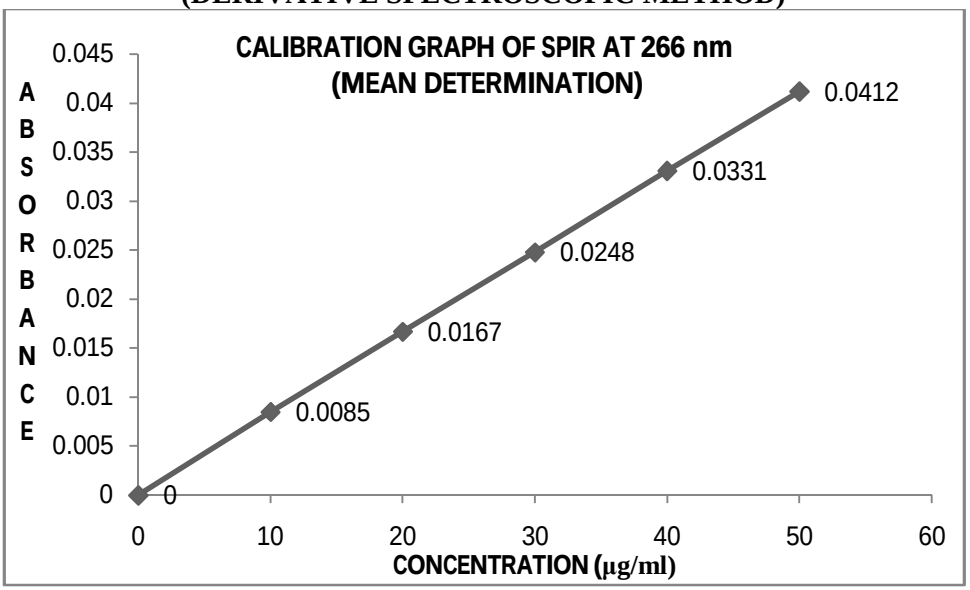
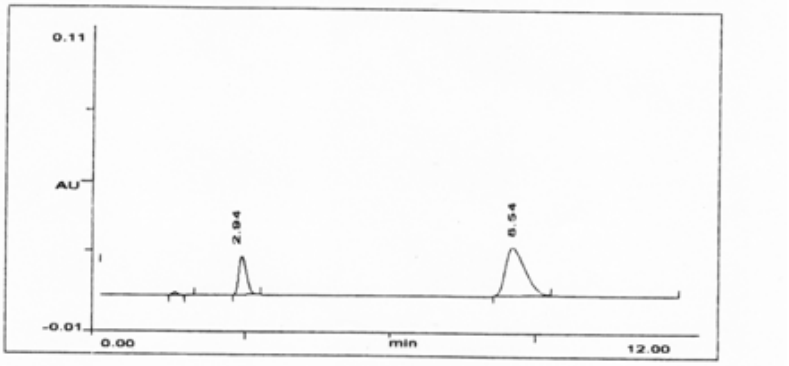


FIGURE-43
LINEARITY CHROMATOGRAM OF METOLAZONE AND SPIRONOLACTONE (0.5, 5 µg ml^{-1})-FIRST SET [1/3]

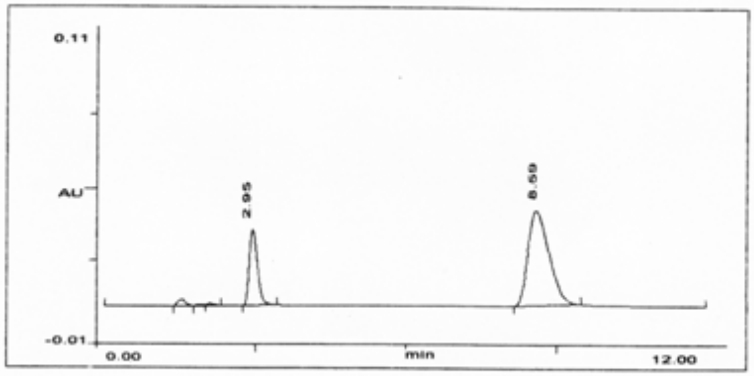
Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	12.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.94	3652	975578	44.8208	24.0626	BB	0.182
2	8.54	4496	3078761	55.1792	75.9374	BB	0.467
		8e+03	4054339				

FIGURE-44
LINEARITY CHROMATOGRAM OF METOLAZONE AND SPIRONOLACTONE (1, 10 µg ml^{-1})- FIRST SET [1/3]

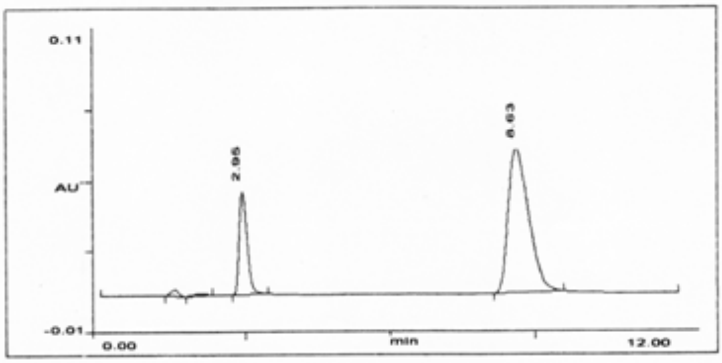
Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	12.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.95	6874	1822142	44.3398	24.0049	BB	0.191
2	8.59	8629	6085144	55.6602	75.9951	BB	0.481
		2e+04	7907286				

FIGURE-45
LINEARITY CHROMATOGRAM OF METOLAZONE AND SPIRONOLACTONE (1.5, 15 µg ml^{-1}) - FIRST SET [1/3]

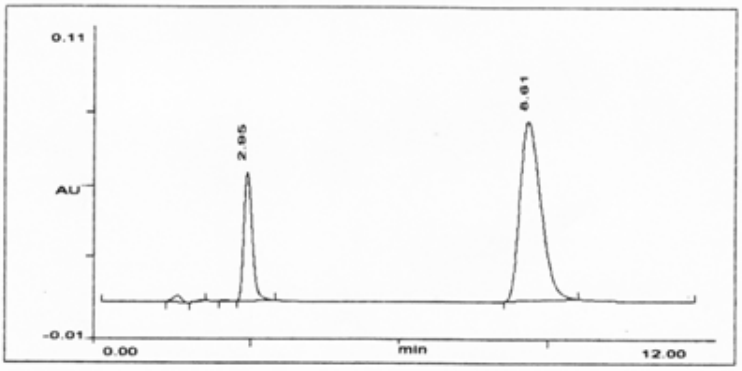
Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	12.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.95	9552	2728740	41.6119	22.1537	BB	0.195
2	8.63	13403	9088598	58.3881	77.8464	BB	0.488
		2e+04	11817338				

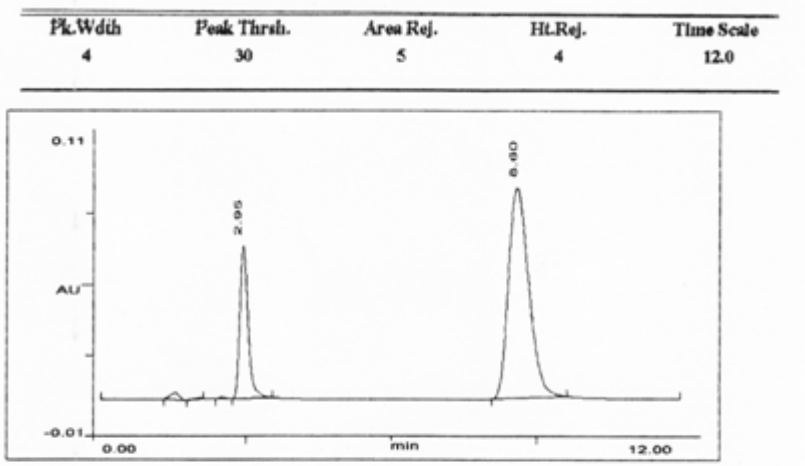
FIGURE-46
LINEARITY CHROMATOGRAM OF METOLAZONE AND SPIRONOLACTONE (2.0, 20 µg ml^{-1})- FIRST SET [1/3]

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	12.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.95	11874	3630620	41.9798	22.6183	BB	0.197
2	8.61	16411	12736834	58.0202	77.3817	BB	0.488
		3e+04	16367454				

FIGURE-47
LINEARITY CHROMATOGRAM OF METOLAZONE AND SPIRONOLACTONE (2.5, 25 µg ml^{-1}) - FIRST SET [1/3]



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.95	14192	4590032	42.0267	23.1183	BB	0.202
2	8.60	19577	15934307	57.9733	76.8817	BB	0.486
		3e+04	20524339				

FIGURE-48
CALIBRATION CURVE OF METOLAZONE BY RP-HPLC

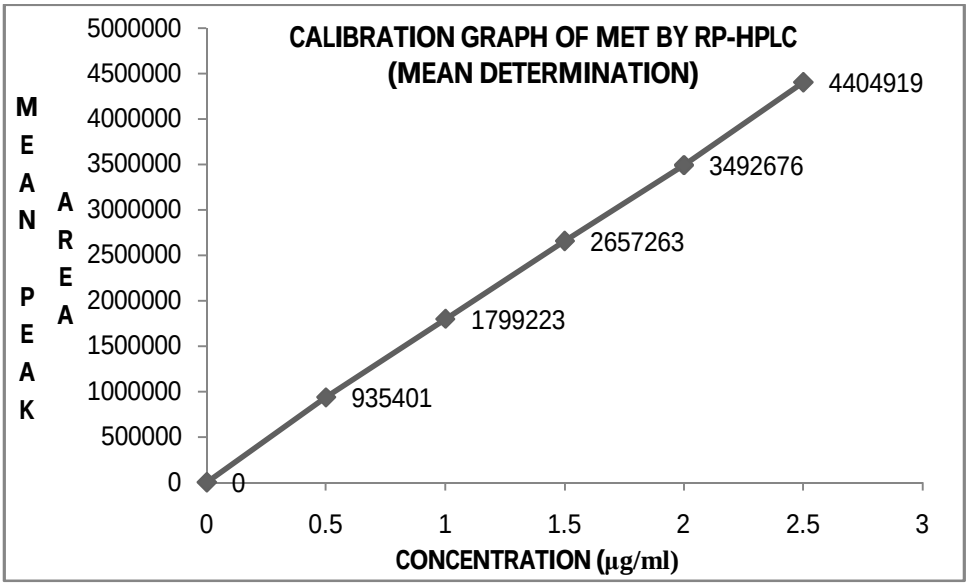


FIGURE-49
CALIBRATION CURVE OF SPIRONOLACTONE
BY RP-HPLC

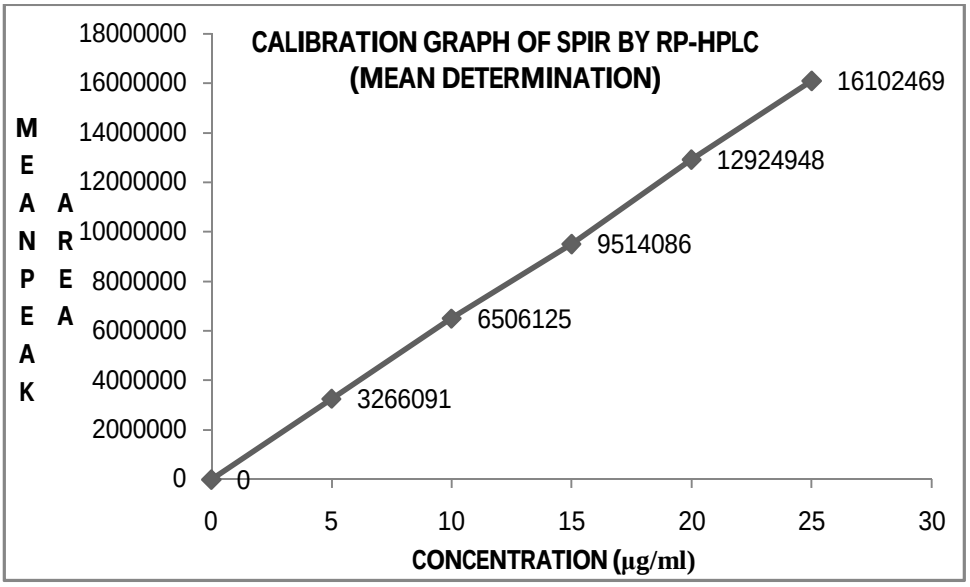
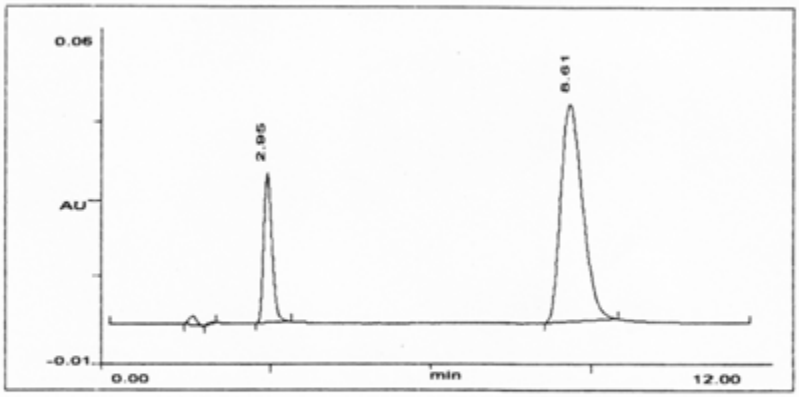


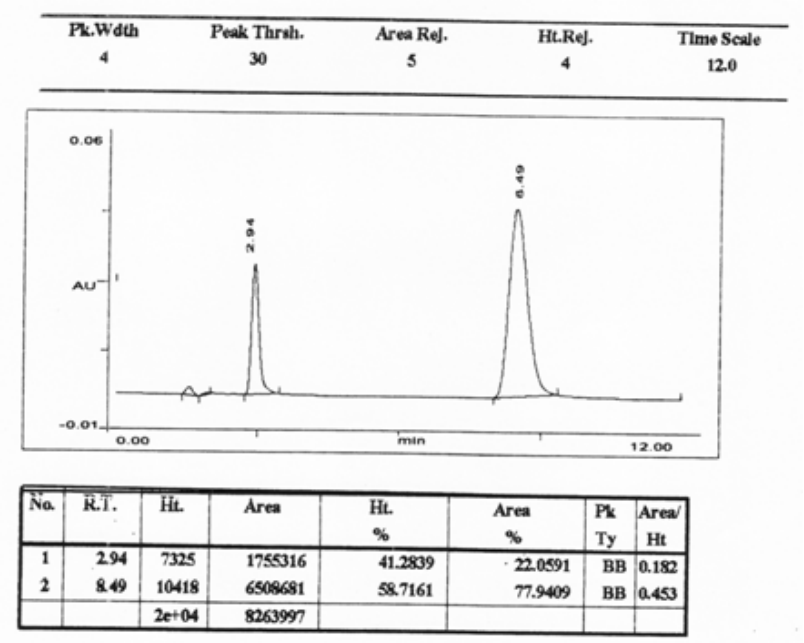
FIGURE-50
CHROMATOGRAM FOR ANALYSIS OF FOMULATION [METOLACTONE-5]
REPEATABILITY -1

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	12.0



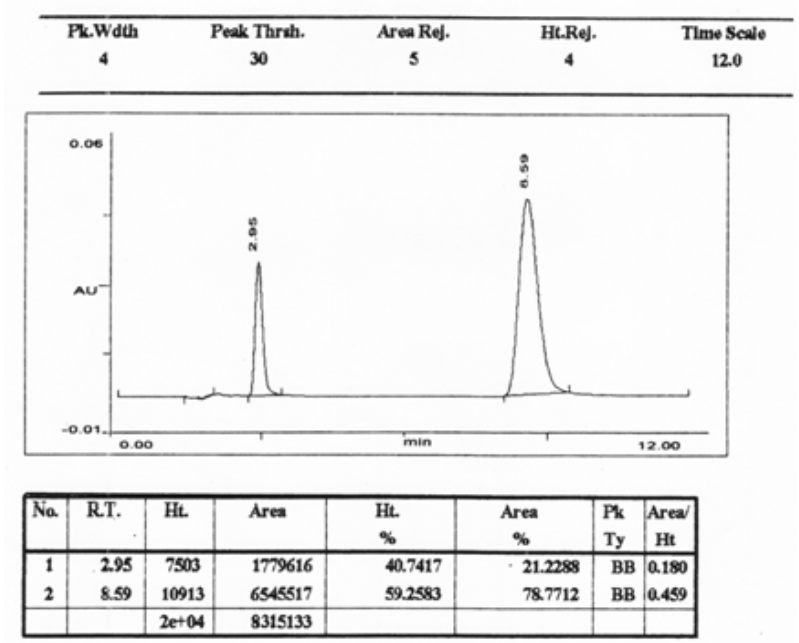
No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.95	7478	1705239	40.8321	21.0889	BB	0.183
2	8.61	10836	6503247	59.1679	78.9111	BB	0.473
		2e+04	8208486				

FIGURE-51
CHROMATOGRAM FOR ANALYSIS OF FORMULATION [METOLACTONE-5]
REPEATABILITY -2



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.94	7325	1755316	41.2839	22.0591	BB	0.182
2	8.49	10418	6508681	58.7161	77.9409	BB	0.453
		2e+04	8263997				

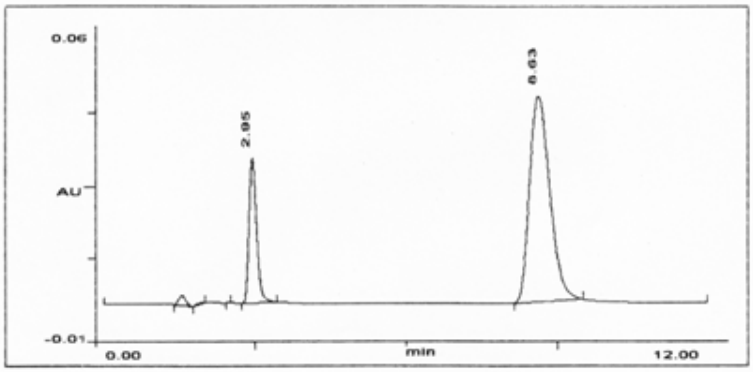
FIGURE-52
CHROMATOGRAM FOR ANALYSIS OF FOMULATION [METOLACTONE-5]
REPEATABILITY -3



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.95	7503	1779616	40.7417	21.2288	BB	0.180
2	8.59	10913	6545517	59.2583	78.7712	BB	0.459
		2e+04	8315133				

FIGURE-53
CHROMATOGRAM FOR ANALYSIS OF FOMULATION [METOLACTONE-5] REPEATABILITY-4

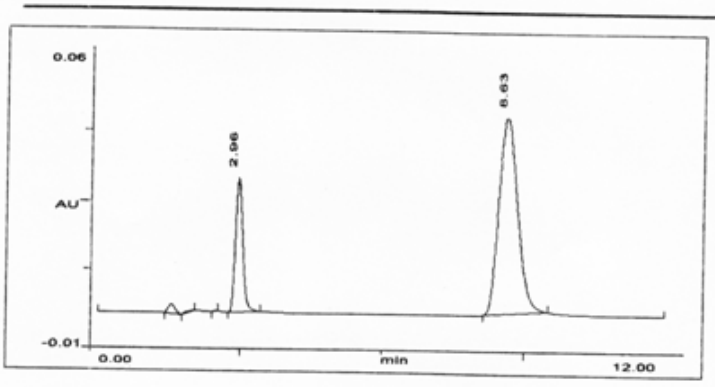
Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	12.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.95	7649	1707538	41.3415	21.4585	BB	0.179
2	8.63	10853	6547897	58.6585	78.5415	BB	0.462
		2e+04	8255435				

FIGURE-54
CHROMATOGRAM FOR ANALYSIS OF FORMULATION [METOLACTONE-5] REPEATABILITY-5

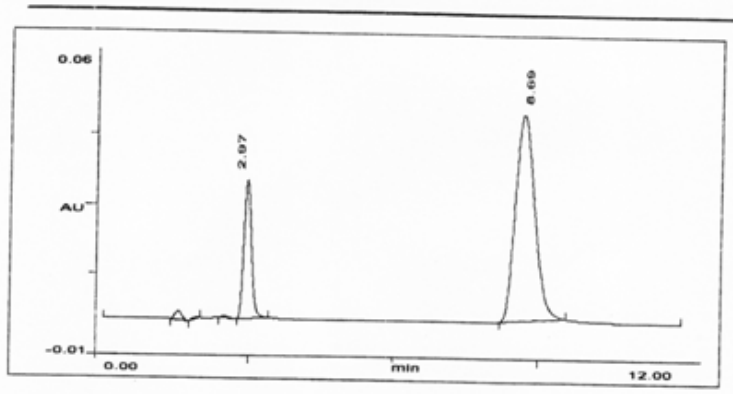
Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	12.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.96	7521	1762444	40.8107	21.1005	BB	0.178
2	8.63	10908	6537996	59.1893	78.8995	BB	0.459
		2e+04	8300440				

FIGURE-55
CHROMATOGRAM FOR ANALYSIS OF FORMULATION [METOLACTONE-5] REPEATABILITY-6

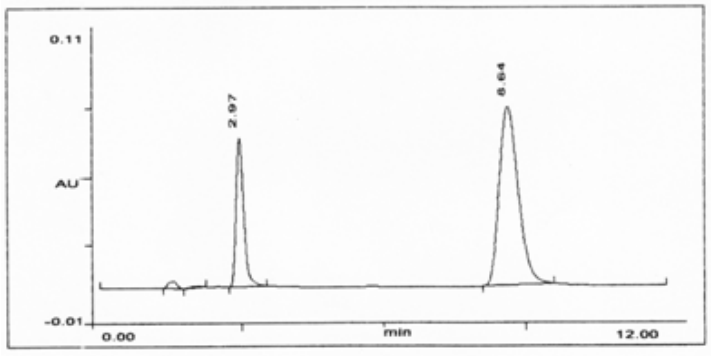
Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	12.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.97	7627	1701495	40.2969	20.7202	BB	0.179
2	8.69	11300	6558155	59.7031	79.2798	BB	0.463
		2e+04	8259650				

FIGURE-56
CHROMATOGRAM FOR 80% RECOVERY OF FORMULATION [METOLACTONE-5]

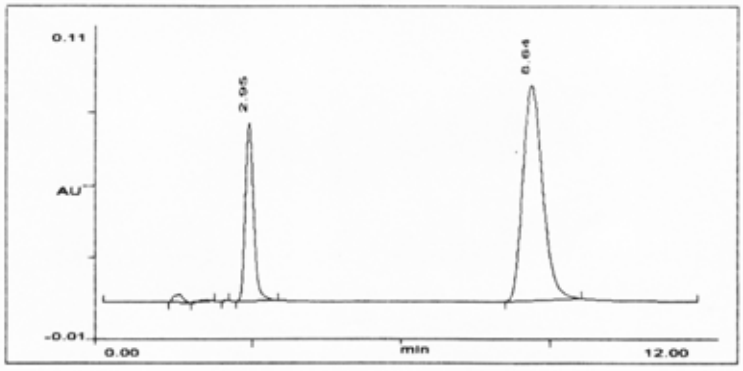
Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	12.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.97	14429	3122544	45.6643	25.7008	BB	0.190
2	8.64	17169	11778880	54.3357	74.2992	BB	0.462
		3e+04	14901424				

FIGURE-57
CHROMATOGRAM FOR 100% RECOVERY OF FORMULATION [METOLACTONE-5]

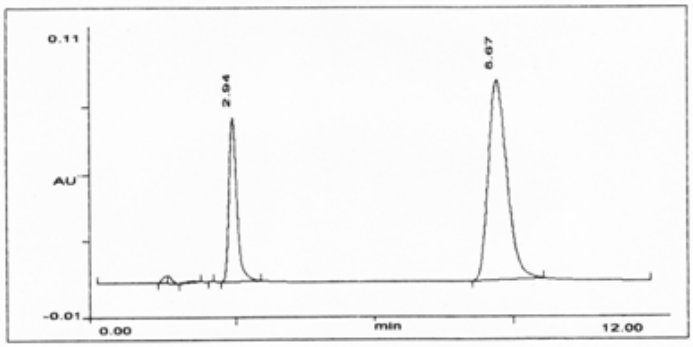
Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	12.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.95	16269	3445916	45.4010	25.9567	BB	0.195
2	8.64	19565	13052830	54.5990	74.0433	BB	0.462
		4e+04	16498746				

FIGURE-58
CHROMATOGRAM FOR 120% RECOVERY OF FORMULATION [METOLACTONE-5]

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	12.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.94	16122	3790946	45.2306	26.1616	BB	0.199
2	8.67	19522	14339722	54.7694	73.8384	BB	0.463
		4e+04	18130668				

FIGURE-59
UV SPECTRUM OF METOPROLOL
IN METHANOL
CONCENTRATION: 10 μg ml^{-1}

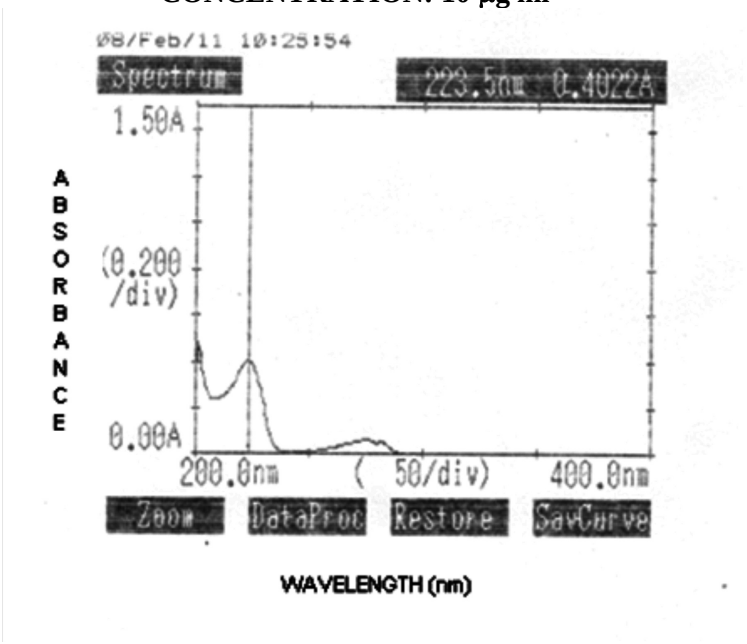


FIGURE-60
UV SPECTRUM OF OLMESARTAN
IN METHANOL
CONCENTRATION: 10 μg ml^{-1}

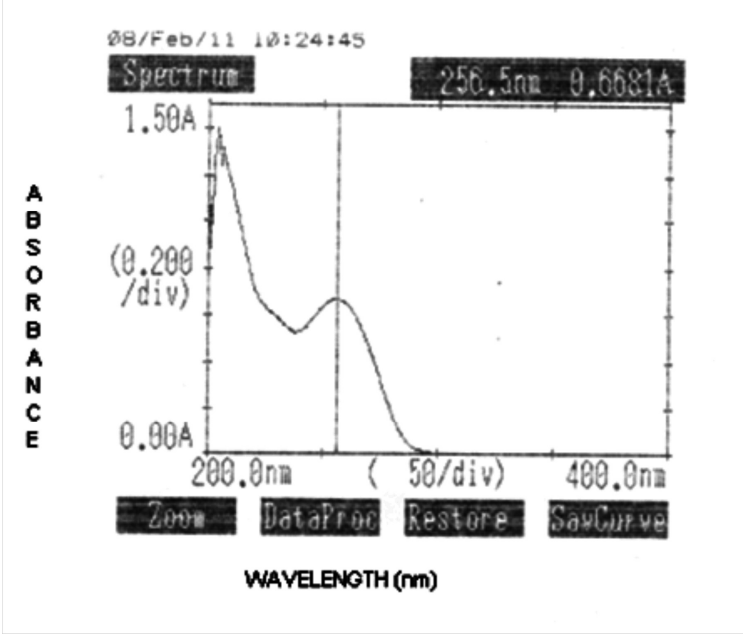


FIGURE-61
OVERLAID SPECTRUM OF METOPROLOL AND OLMESARTAN IN METHANOL

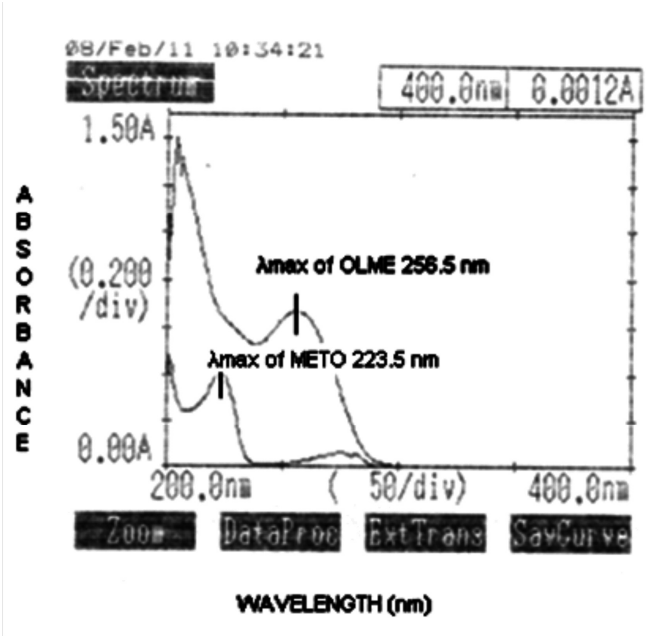


FIGURE-62
CALIBRATION CURVE OF METOPROLOL IN METHANOL AT 223.5 nm

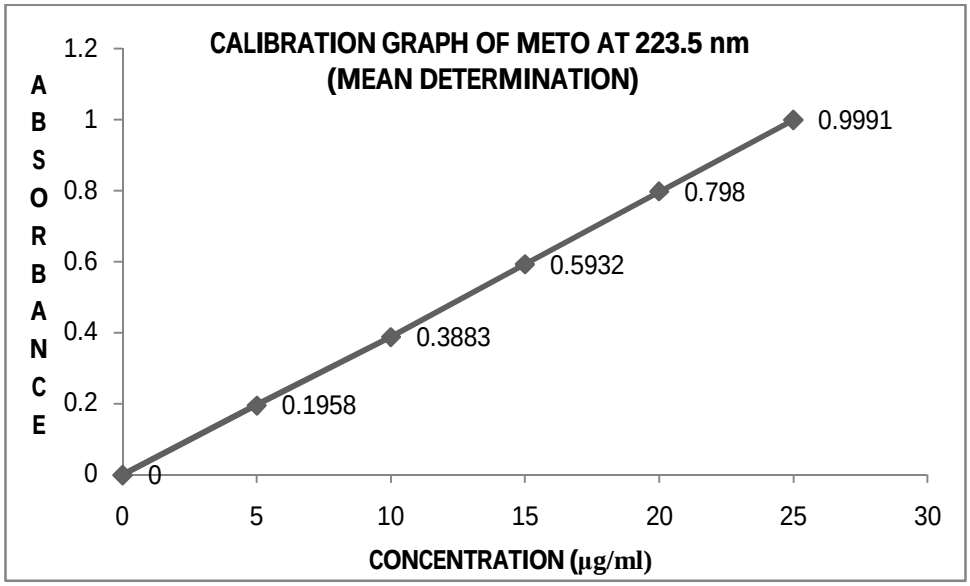


FIGURE-63
CALIBRATION CURVE OF METOPROLOL
IN METHANOL AT 256.5 nm

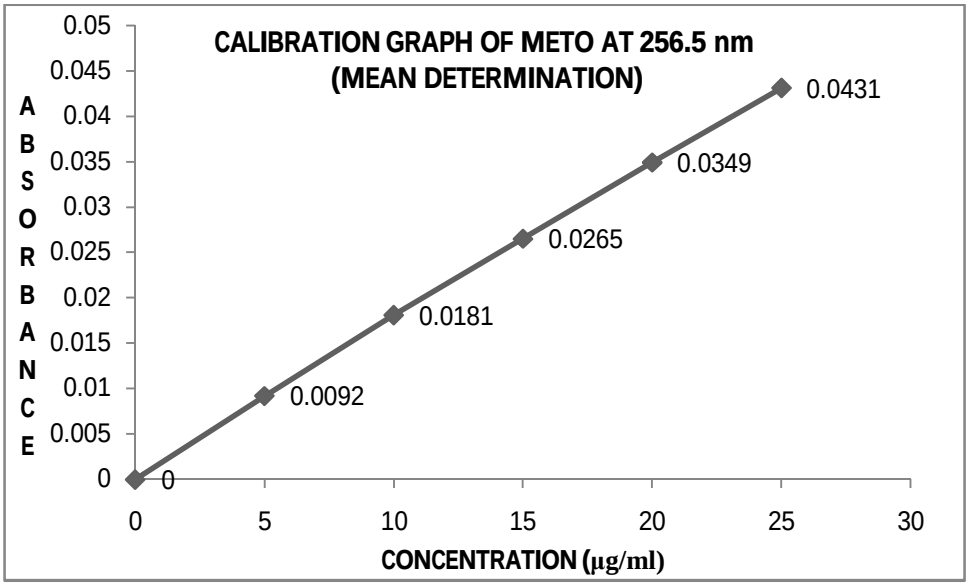


FIGURE-64
CALIBRATION CURVE OF OLMESARTAN
IN METHANOL AT 223.5 nm

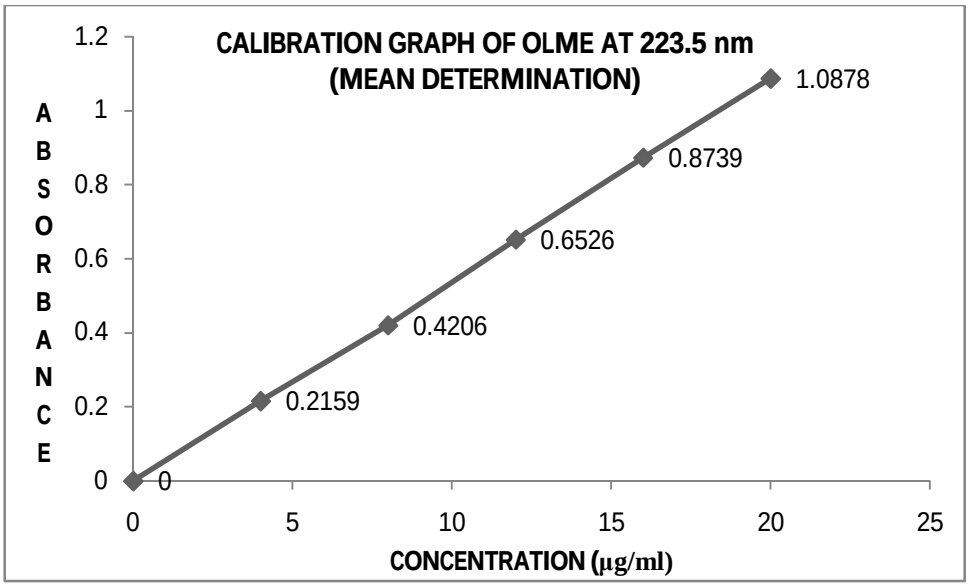


FIGURE-65
CALIBRATION CURVE OF OLMESARTAN
IN METHANOL AT 256.5 nm

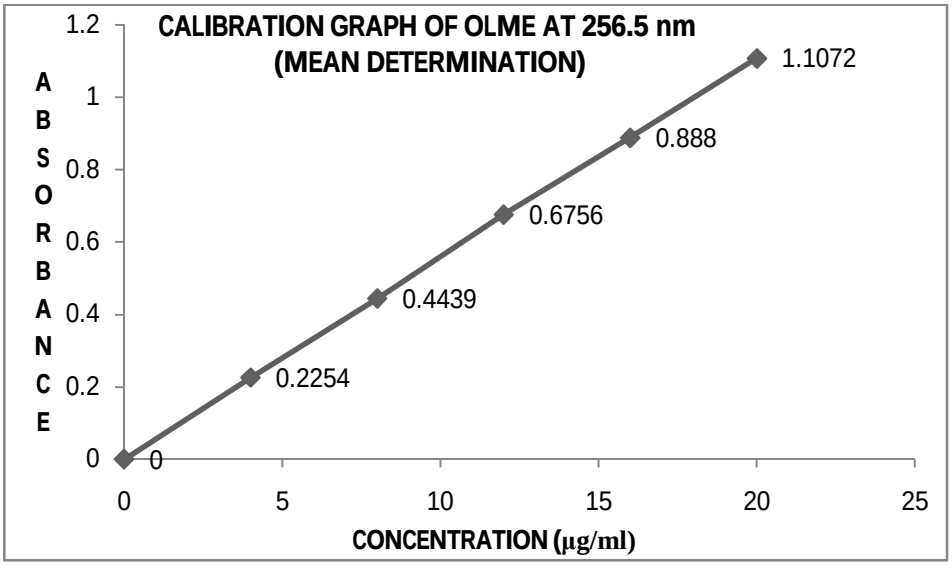


FIGURE-66
OVERLAID SPECTRUM OF METOPROLOL AND OLMESARTAN
IN METHANOL
[AREA UNDER THE CURVE METHOD]

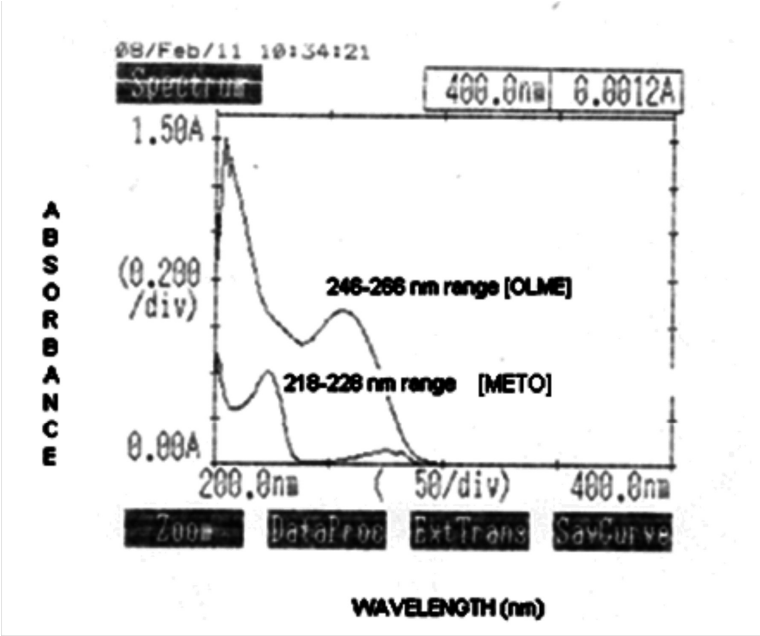


FIGURE-67
CALIBRATION CURVE OF METOPROLOL
IN METHANOL AT 218-228 nm
[AREA UNDER THE CURVE METHOD]

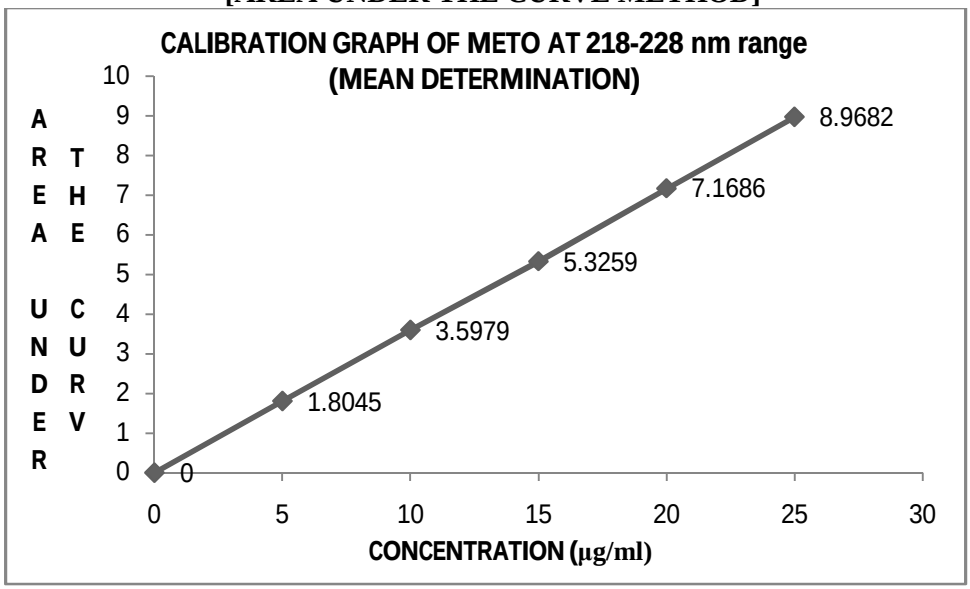


FIGURE-68
CALIBRATION CURVE OF METOPROLOL
IN METHANOL AT 246-266 nm
[AREA UNDER THE CURVE METHOD]

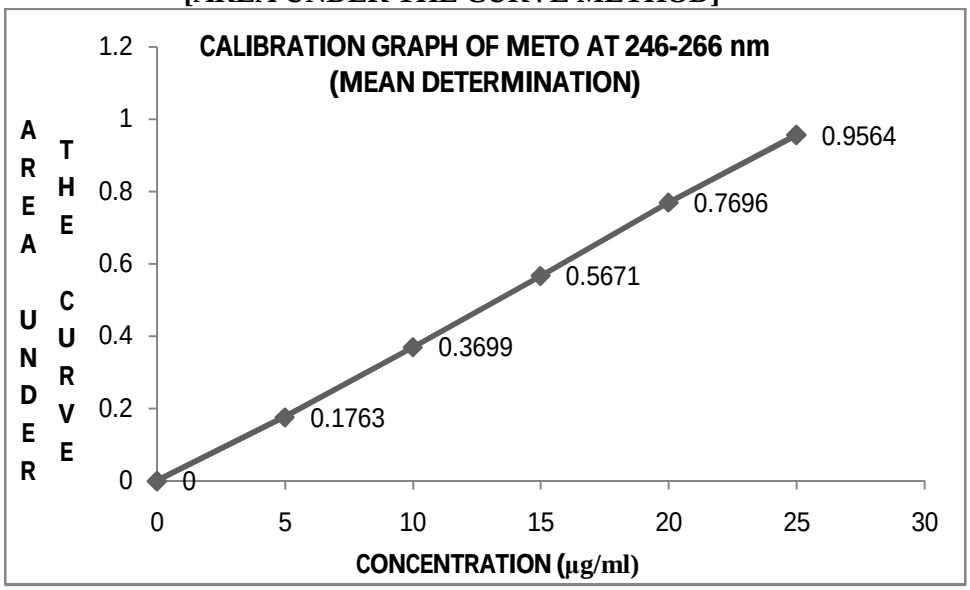


FIGURE-69
CALIBRATION CURVE OF OLMESARTAN
IN METHANOL AT 218-228 nm
[AREA UNDER THE CURVE METHOD]

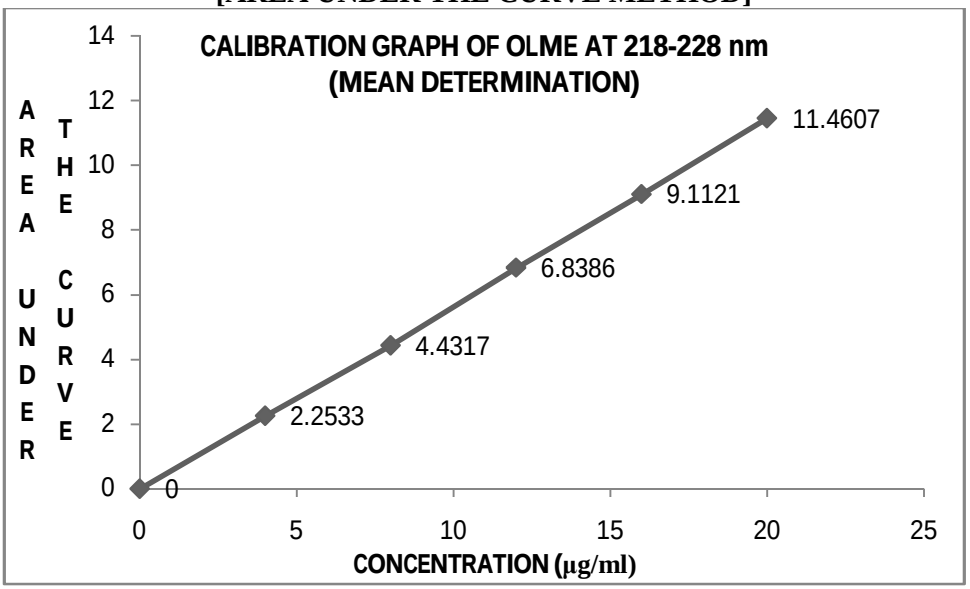


FIGURE-70
CALIBRATION CURVE OF OLMESARTAN
IN METHANOL AT 246-266 nm
[AREA UNDER THE CURVE METHOD]

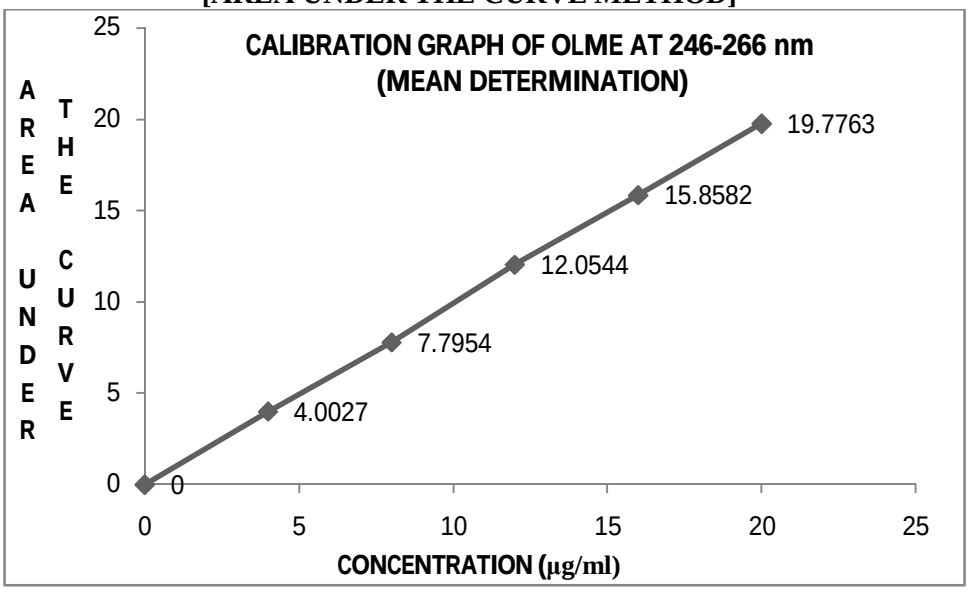


FIGURE-71
FIRST ORDER DERIVATIVE UV SPECTRUM OF METOPROLOL IN METHANOL
(DERIVATIVE SPECTROSCOPIC METHOD)

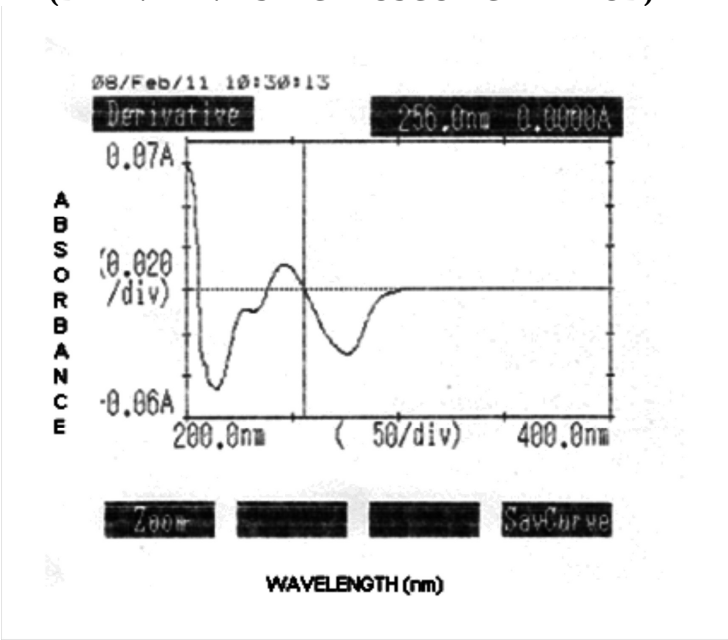


FIGURE-72
FIRST ORDER DERIVATIVE UV SPECTRUM OF OLMESARTAN IN METHANOL
(DERIVATIVE SPECTROSCOPIC METHOD)

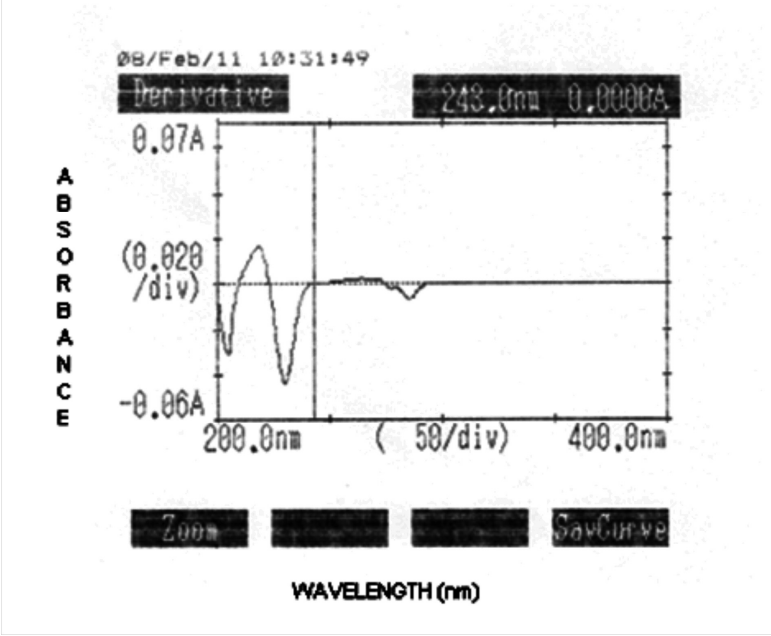


FIGURE-73
OVERLAID FIRST ORDER DERIVATIVE SPECTRUM OF METOPROLOL AND OLMESARTAN IN METHANOL
(DERIVATIVE SPECTRSCOPIC METHOD)

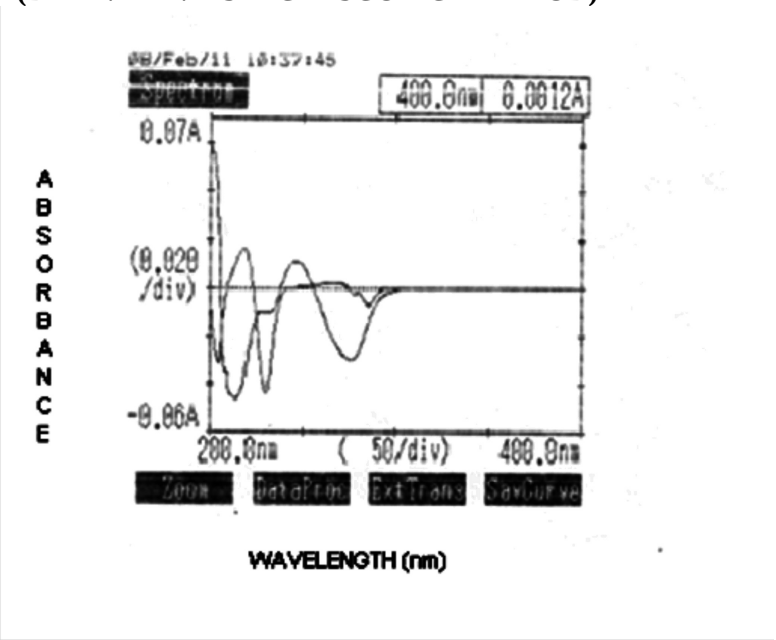


FIGURE-74
CALIBRATION CURVE OF METOPROLOL AT 256 nm
(DERIVATIVE SPECTROSCOPIC METHOD)

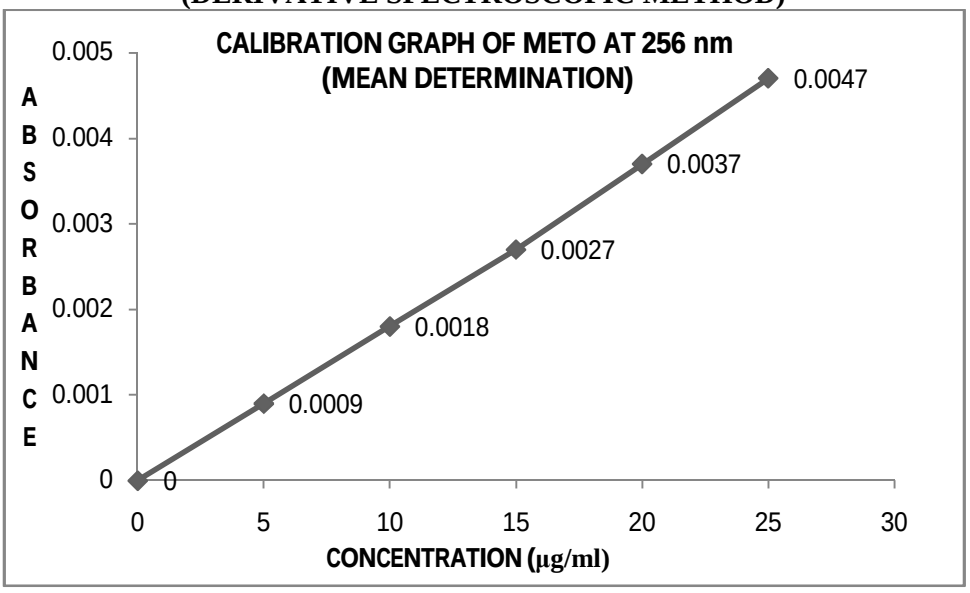


FIGURE-75
CALIBRATION CURVE OF OLMESARTAN
AT 243 nm
(DERIVATIVE SPECTROSCOPIC METHOD)

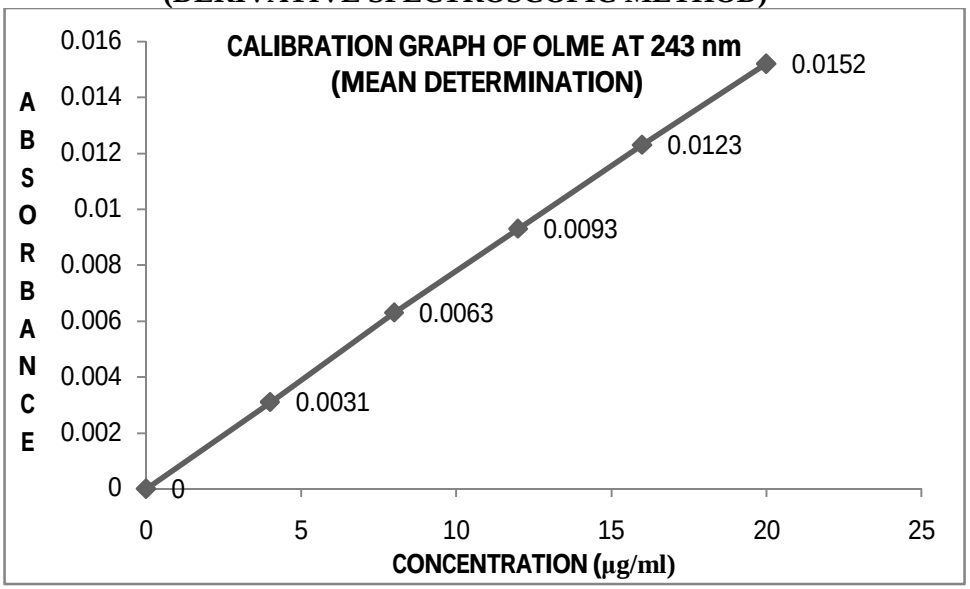


FIGURE-76
UV SPECTRUM OF ASPIRIN
IN METHANOL
CONCENTRATION: 10 µg ml^{-1}

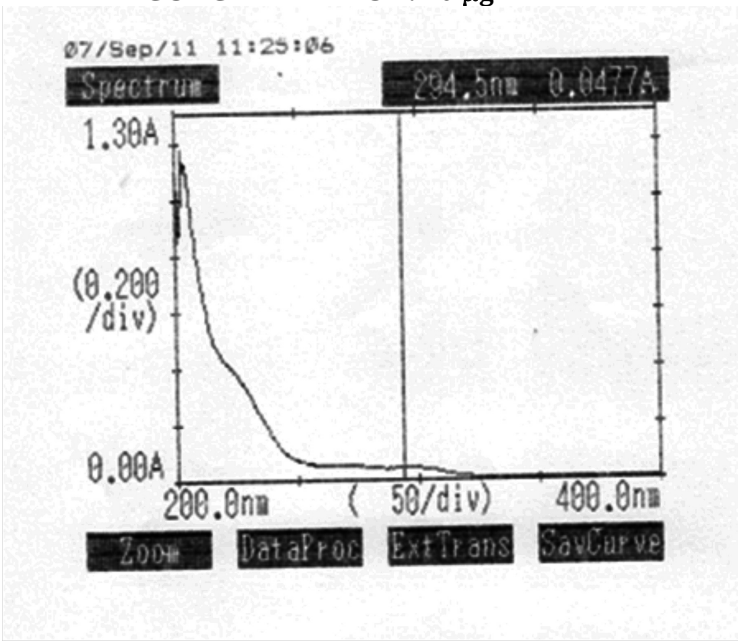


FIGURE-77
UV SPECTRUM OF ROSUVASTATIN
IN METHANOL
CONCENTRATION: 10 μg ml^{-1}

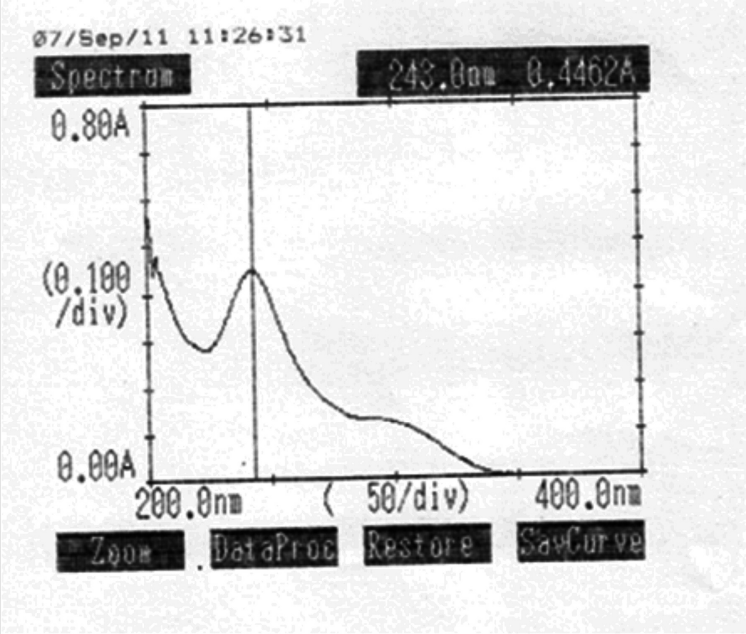


FIGURE-78
OVERLAID SPECTRUM OF ASPIRIN AND ROSUVASTATIN
IN METHANOL

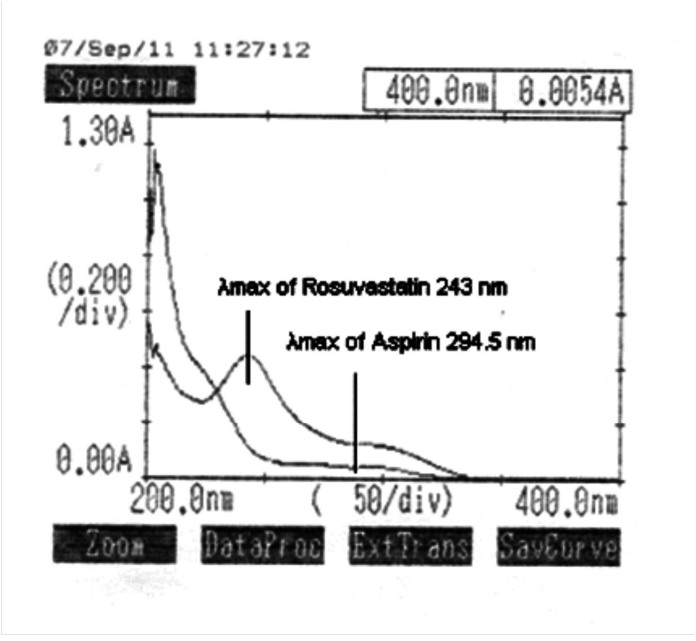


FIGURE-79
CALIBRATION CURVE OF ASPIRIN
IN METHANOL AT 294.5 nm

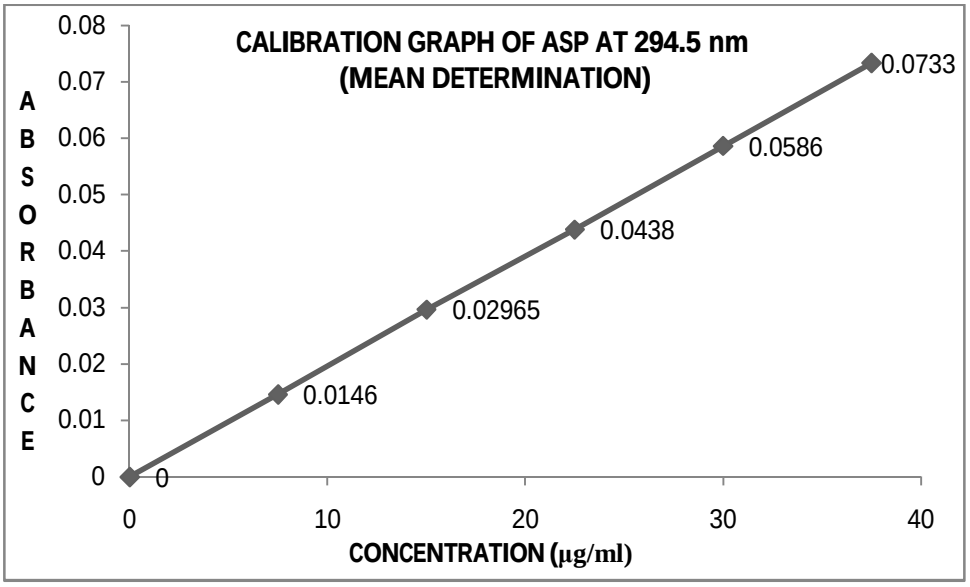


FIGURE-80
CALIBRATION CURVE OF ASPIRIN
IN METHANOL AT 243 nm

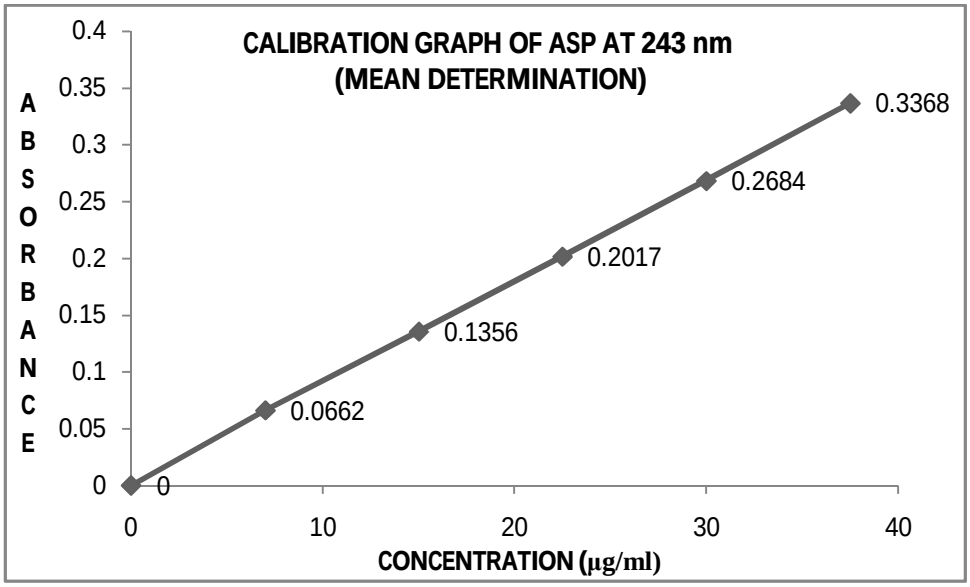


FIGURE-81
CALIBRATION CURVE OF ROSUVASTATIN IN METHANOL AT 294.5 nm

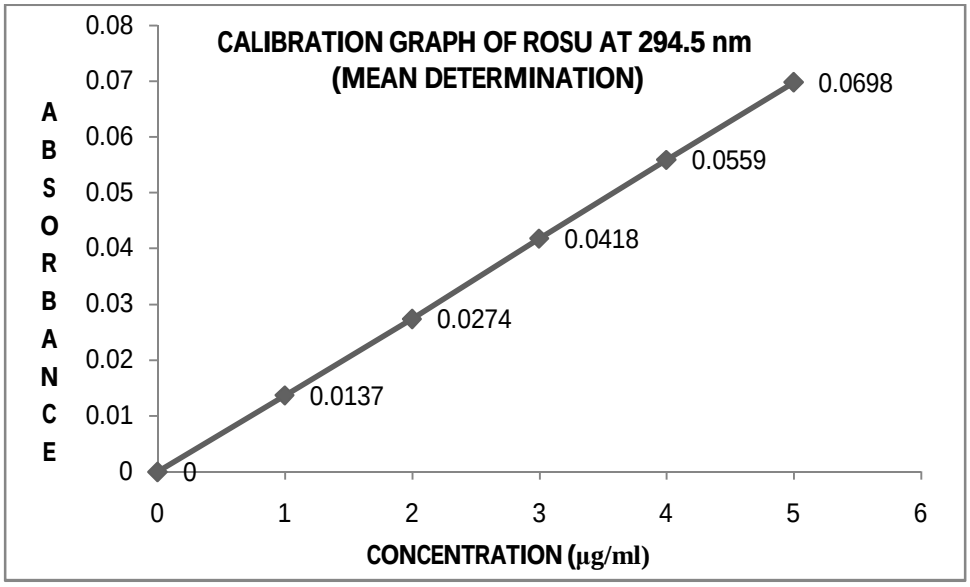


FIGURE-82
CALIBRATION CURVE OF ROSUVASTATIN IN METHANOL AT 243 nm

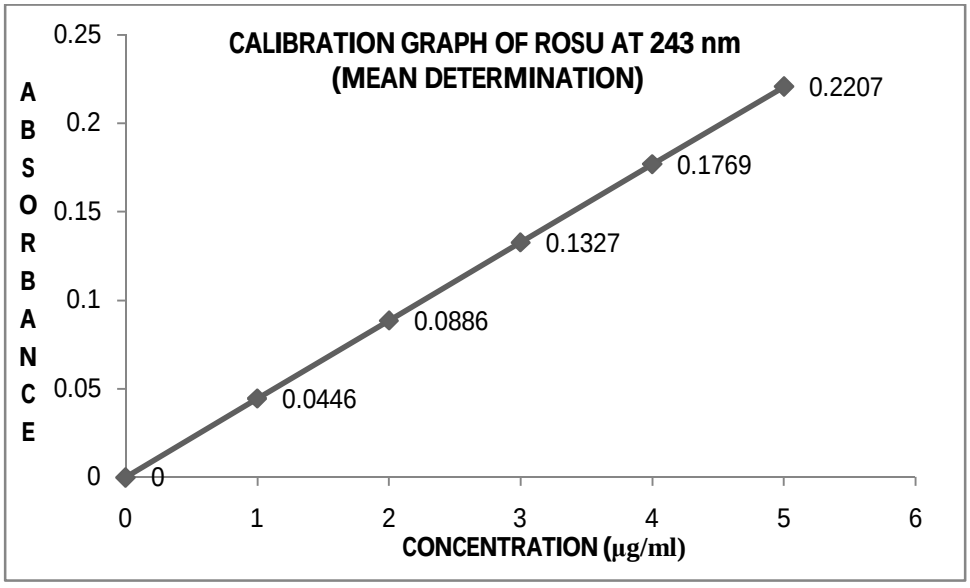


FIGURE-83
OVERLAID SPECTRUM OF ASPIRIN AND ROSUVASTATIN
(ABSORBANCE RATIO METHOD)

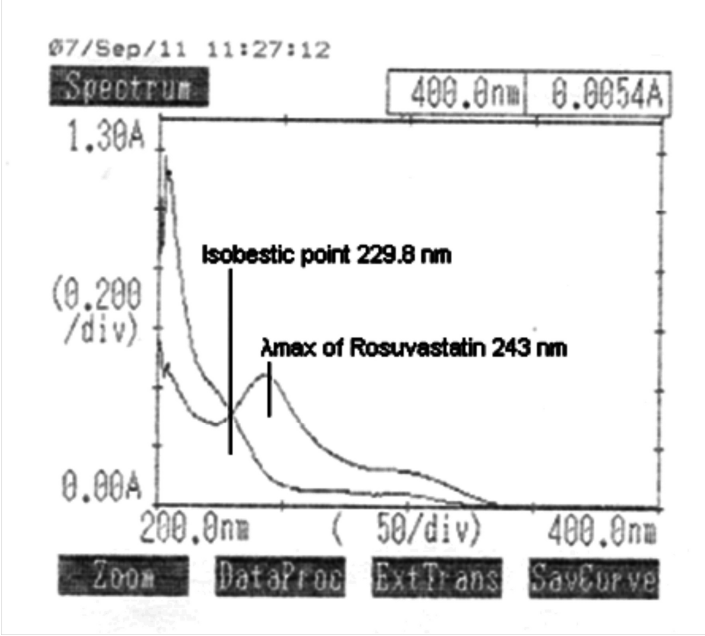


FIGURE-84
CALIBRATION CURVE OF ASPIRIN
IN METHANOL AT 229.8 nm

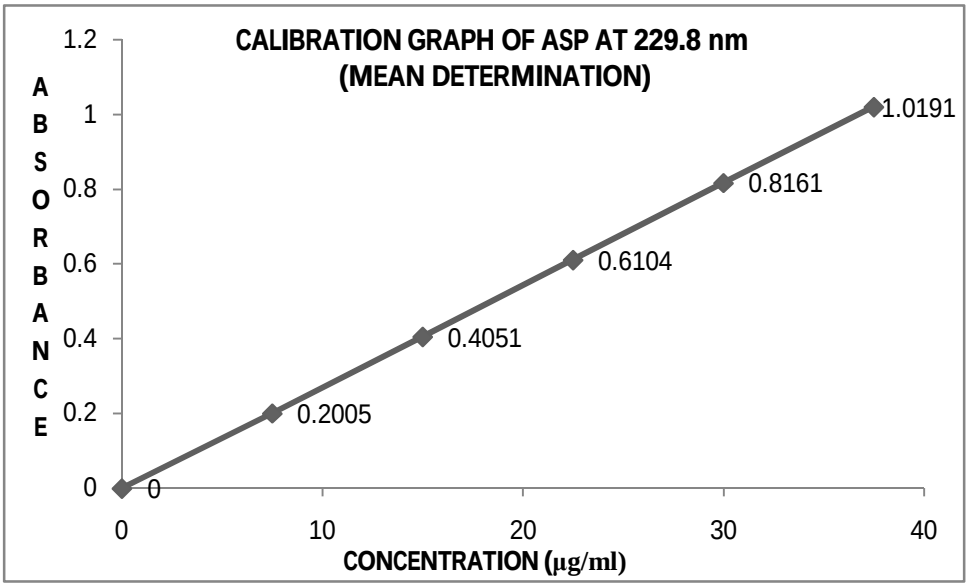


FIGURE-85
CALIBRATION CURVE OF ROSUVASTATIN IN METHANOL AT 229.8 nm

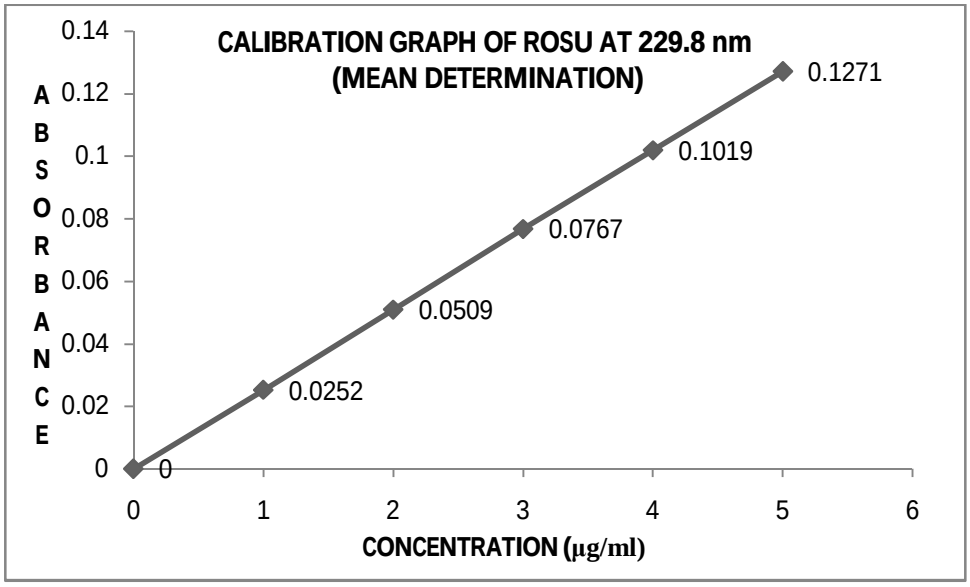


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TABLE-1

SOLUBILITY PROFILE OF DOXOFYLLINE AND AMBROXOL HYDROCHLORIDE IN POLAR AND NON POLAR SOLVENTS

S.NO	SOLVENTS	DOXOFYLLINE	AMBROXOL HYDROCHLORIDE
1	Distilled Water	Soluble	Sparingly Soluble
2	0.1M Sodium hydroxide	Sparingly Soluble	Insoluble
3	0.1 M Hydrochloric acid	Soluble	Slightly Soluble
4	Methanol	Soluble	Freely soluble
5	Acetone	Soluble	Insoluble
6	Acetonitrile	Freely Soluble	Slightly soluble
7	Ethanol	Sparingly Soluble	Slightly soluble
8	Chloroform	Soluble	Insoluble
9	Dimethyl formamide	Freely Soluble	Very Slightly Soluble
10	Isopropyl alcohol	Insoluble	Insoluble
11	Benzene	-	Insoluble
12	n-butanol	-	Very Slightly Soluble
13	Dichloroethane	Very Freely Soluble	Very Slightly Soluble
14	Diethyl ether	Slightly Soluble	Insoluble
15	Ethyl acetate	Sparingly Soluble	Insoluble
16	Cyclohexane	Sparingly Soluble	Insoluble
17	Pyridine	-	Soluble
18	Petroleum ether	Insoluble	-
19	Toluene	Sparingly Soluble	-
20	n-hexane	Insoluble	-
21	Carbon tetrachloride	Sparingly Soluble	-

TABLE-2

STABILITY STUDY OF DOXOFYLLINE AND AMBROXOL HYDROCHLORIDE FOR UV SPECTROSCOPIC METHODS

Solvent: Distilled Water

Concentration of Ambroxol Hydrochloride and Doxofylline: 10 µg/ml

S.No	Time	Absorbance of Doxofylline (274 nm)	Absorbance of Ambroxol Hydrochloride (244.5 nm)
1	0 min	0.348	0.252
2	10 min	0.347	0.250
3	20 min	0.350	0.254
4	30 min	0.351	0.256
5	40 min	0.354	0.253
6	50 min	0.353	0.255
7	60 min	0.349	0.252
8	1 hour 15 min	0.350	0.252
9	1 hour 30 min	0.348	0.253
10	1 hour 45 min	0.348	0.251
11	2 hours	0.350	0.249
12	2 hours 30 min	0.351	0.247
13	3 hours	0.351	0.253
14	3 hours 30 min	0.349	0.254
15	4 hours	0.352	0.253
16	24 hours	0.349	0.247

TABLE-3
OPTICAL CHARACTERISTICS OF DOXOFYLLINE
(SIMULTANEOUS EQUATION METHOD)

PARAMETERS	AT 274 nm*	AT 244.5 nm*
Beer's law limit (µg mL^{-1})	7-35 (µg mL^{-1})	7-35 (µg mL^{-1})
Molar absorptivity	9330.73755	2374.305627
Sandells sensitivity (µg/cm^2/0.001 A.U)	0.02872	0.11104
Correlation coefficient (r)	0.999970	0.999390
Regression equation (y= mx + c)	y= (0.03483)x + (0.00209)	y= (0.00901)x + (-0.00093)
Slope (m)	0.03483	0.00901
Intercept (c)	0.00209	-0.00093
LOD (µg mL^{-1})	0.216615	0.829938
LOQ (µg mL^{-1})	0.656410	2.51496
Standard error	0.0004651	0.0014219

*Mean of six observations

TABLE-4
OPTICAL CHARACTERISTICS OF AMBROXOL HYDROCHLORIDE
(SIMULTANEOUS EQUATION METHOD)

PARAMETERS	AT 244.5 nm*	AT 274 nm*
Beer's law limit (µg mL^{-1})	1-5 (µg mL^{-1})	1-5 (µg mL^{-1})
Molar absorptivity	14467.40119	1725.394095
Sandell's sensitivity (µg/cm^2/ 0.001 A.U)	0.029669	0.302388817
Correlation coefficient (r)	0.999413	0.991096
Regression equation (y= mx + c)	y = (0.03478)x +0.001124	y = (0.00409)x + 0.00062
Slope (m)	0.03478	0.00409
Intercept (c)	0.001124	0.00062
LOD (µg mL^{-1})	0.129641	1.246725
LOQ (µg mL^{-1})	0.392853	3.777957
Standard error	0.0002335	0.000230

*****Mean of six observations

TABLE-5
QUANTIFICATION OF FORMULATION [SYNASMA-AX]
(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	399.81	99.95	99.97	0.32750	0.0818	0.13370
	2	400	400.04	100.01				
	3	400	399.56	99.89				
	4	400	400.21	100.05				
	5	400	399.50	99.87				
	6	400	400.27	100.06				
AMB	1	30	29.45	98.16	98.64	0.13841	0.46779	0.05651
	2	30	29.52	98.42				
	3	30	29.71	99.03				
	4	30	29.52	98.42				
	5	30	29.81	99.37				
	6	30	29.53	98.42				

*****Mean of six observations

TABLE-6
INTRADAY AND INTERDAY ANALYSIS OF FORMULATION [SYNASMA-AX]
(SIMULTANEOUS EQUATION METHOD)f

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.	
			Intraday	Interday	Intraday	Interday	Intraday	Interday
DOX	1	400	99.94	99.95	0.25520	0.290	0.0638	0.0726
	2	400	99.91	99.98				
	3	400	99.84	100.02				
	4	400	99.87	99.86				
	5	400	99.87	99.97				
	6	400	99.85	99.875				
Mean			99.88	99.94				
AMB	1	30	98.71	98.84	0.02888	0.29017	0.097	0.16348
	2	30	98.77	98.84				
	3	30	98.68	98.84				
	4	30	98.88	99.13				
	5	30	98.83	99.10				
	6	30	98.67	98.88				
Mean			98.75	98.94				

*Mean of three observations

TABLE-7
RUGGEDNESS STUDY OF FORMULATION
[SYNASMA-AX]
(SIMULTANEOUS EQUATION METHOD)

Drug	Condition	Average* % Obtained	S.D	% R.S.D	S.E.
DOX	Analyst 1	99.88	0.38557	0.09651	0.15741
	Analyst 2	99.89	0.52015	0.13017	0.21235
	Instrument 1	99.66	0.39635	0.09929	0.161811
	Instrument 2	99.97	0.32734	0.08200	0.13366
AMB	Analyst 1	98.91	0.06969	0.23489	0.02845
	Analyst 2	98.68	0.07711	0.26049	0.03148
	Instrument 1	98.68	0.07893	0.26661	0.03222
	Instrument 2	98.64	0.13851	0.46701	0.05655

*****Mean of six observations

TABLE-8
RECOVERY STUDY DATA OF 50% PRE-ANALYSED FORMULATION
[SYNASMA-AX]
(SIMULTANEOUS EQUATION METHOD)

Drug	Percentage	Amount present* ($\mu g\ ml^{-1}$)	Amount added* ($\mu g\ ml^{-}$)	Amount estimated* ($\mu g\ ml^{-1}$)	Amount recovered* ($\mu g\ ml^{-1}$)	% Recovery*	S.D.	% RSD	S.E.
DOX	80	13.9979	11.2	25.2058	11.2079	100.087	0.01126	0.10046	0.00650
	100	13.9979	14	27.9951	13.9971	99.98	0.01214	0.08673	0.07009
	120	13.9979	16.8	30.8028	16.8049	100.023	0.00081	0.00482	0.00047
AMB	80	1.013	0.8	1.80036	0.7874	98.417	0.00120	0.15240	0.00069
	100	1.013	1	2.0116	0.9998	99.86	0.00365	0.36507	0.21073
	120	1.013	1.2	2.2065	1.1935	99.4567	0.0021	0.18430	0.00121

*Mean of three observations

TABLE-9
OPTICAL CHARACTERISTICS OF DOXOFYLLINE
(ABSORBANCE CORRECTION METHOD)

PARAMETERS	AT 274 nm*
Beer's law limit (µg mL^{-1})	7-35 (µg mL^{-1})
Molar absorptivity	9330.73755
Sandell' s sensitivity (µg/cm^2/0.001 A.U)	0.02872
Correlation coefficient (r^2)	0.999970
Regression equation (y= mx + c)	y= (0.03483)x + (0.00209)
Slope (m)	0.03483
Intercept (c)	0.00209
LOD (µg mL^{-1})	0.216615
LOQ (µg mL^{-1})	0.656410
Standard error	0.0004651

*Mean of six observations

TABLE-10
OPTICAL CHARACTERISTICS OF AMBROXOL HYDROCHLORIDE
(ABSORBANCE CORRECTION METHOD)

PARAMETERS	AT 274 nm*	AT 308 nm*
Beer's law limit (µg mL^{-1})	1-5 (µg mL^{-1})	1-5 (µg mL^{-1})
Molar absorptivity	1725.394095	4868.786
Sandell's sensitivity (µg/cm^2/0.001 A.U)	0.302388817	0.08848
Correlation coefficient (r)	0.991096	0.99933
Regression equation (y = mx + c)	y = (0.00409)x + 0.00062	y = 0.011726x + 0.000166
Slope (m)	0.00409	0.011726
Intercept (c)	0.00062	0.000166
LOD (µg mL^{-1})	1.246725	0.053809
LOQ (µg mL^{-1})	3.777957	0.163058
Standard error	0.000230	9.37974 E-05

*****Mean of six observations

TABLE-11
QUANTIFICATION OF FORMULATION [SYNASMA-AX]
(ABSORBANCE CORRECTION 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	403.06	100.76	100.32	2.0275	0.50529	0.82775
	2	400	402.08	100.52				
	3	400	398.96	99.74				
	4	400	401.17	100.29				
	5	400	403.55	100.88				
	6	400	398.79	99.70				
AMB	1	30	30.04	100.14	99.60	0.19595	0.65582	0.08
	2	30	29.80	99.33				
	3	30	30.04	100.14				
	4	30	29.56	98.53				
	5	30	29.80	99.33				
	6	30	30.04	100.13				

*****Mean of six observations

TABLE-12
INTRADAY AND INTERDAY ANALYSIS OF FORMULATION [SYNASMA-AX]
(ABSORBANCE CORRECTION METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.	
			Intraday	Interday	Intraday	Interday	Intraday	Interday
DOX	1	400	100.05	100.16	0.66545	0.42507	0.16602	0.10613
	2	400	100.20	100.12				
	3	400	100.18	100.17				
	4	400	100.18	100.14				
	5	400	100.36	99.99				
	6	400	100.28	100.09				
Mean			100.21	100.11				
AMB	1	30	98.78	99.71	0.17418	0.18831	0.58398	0.62845
	2	30	99.60	99.71				
	3	30	99.93	99.71				
	4	30	99.87	99.30				
	5	30	99.59	99.31				
	6	30	99.33	99.30				
Mean			99.51	99.51				

*Mean of three observations

TABLE-13
RUGGEDNESS STUDY OF FORMULATION [SYNASMA-AX]
(ABSORBANCE CORRECTION METHOD)

Drug	Condition	Average* % Obtained	S.D	% R.S.D	S.E.
DOX	Analyst 1	99.95	0.54375	0.13601	0.22199
	Analyst 2	99.99	0.60929	0.15235	0.24874
	Instrument 1	99.89	0.36348	0.09097	0.14839
	Instrument 2	100.32	0.50671	0.5051	0.20686
AMB	Analyst 1	99.30	0.21466	0.72059	0.08764
	Analyst 2	99.30	0.21466	0.72059	0.08764
	Instrument 1	99.03	0.19596	0.65957	0.07999
	Instrument 2	99.60	0.19515	0.6550	0.07966

*****Mean of six observations

TABLE-14
RECOVERY STUDY DATA OF 50% PRE-ANALYSED FORMULATION [SYNASMA-AX] (ABSORBANCE CORRECTION METHOD)

Drug	Percentage	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.
DOX	80	14.0471	11.2	25.24457	11.19747	99.97	0.00438	0.03907	0.00253
	100	14.0471	14	27.99667	13.94957	99.64	0.00443	0.03175	0.00256
	120	14.0471	16.8	30.84827	16.80117	100.00	0.00875	0.05208	0.00505
AMB	80	1.0460	0.8	1.8504	0.80443	100.55	0.00495	0.61534	0.00286
	100	1.0460	1.0	2.04373	1.00057	100.06	0.00491	0.49072	0.00283
	120	1.0460	1.2	2.24277	1.19677	99.73	0.00491	0.41027	0.00284

*Mean of three observations

TABLE-15
OPTICAL CHARACTERISTICS OF DOXOFYLLINE
(ABSORBANCE RATIO METHOD)

PARAMETERS	AT 233.5 nm*	AT 244.5 nm*
Beer's law limit ($\mu g\ ml^{-1}$)	7-35 ($\mu g\ mL^{-1}$)	7-35 ($\mu g\ mL^{-1}$)
Molar absorptivity	4930.64917	2374.305627
Sandell's sensitivity ($\mu g/cm^2$/0.001 A.U)	0.054045077	0.111044398
Correlation coefficient (r^2)	0.999916585	0.999390328
Regression equation (y= mx + c)	y = (0.018514)x + (3.88889 E-05)	y = (0.00901034)x + (-0.000930952)
Slope (m)	0.018514	0.00901034
Intercept (c)	3.88889 E-05	-0.000930952
LOD ($\mu g\ ml^{-1}$)	0.466573211	0.829938025
LOQ ($\mu g\ ml^{-1}$)	1.413858215	2.514963713
Standard error	0.000632051	0.001421926

*Mean of six observations

TABLE-16
OPTICAL CHARACTERISTICS OF AMBROXOL HYDROCHLORIDE
(ABSORBANCE RATIO METHOD)

PARAMETERS	AT 233.5 nm*	AT 244.5 nm*
Beer's law limit ($\mu g\ mL^{-1}$)	1-5 ($\mu g\ mL^{-1}$)	1-5 ($\mu g\ mL^{-1}$)
Molar absorptivity	12853.45552	14467.40119
Sandell's sensitivity ($\mu g/cm^2$/0.001 A.U)	0.033610515	0.029669537
Correlation coefficient (r^2)	0.999299449	0.999413463
Regression equation (y = mx + c)	y = (0.030895714)x + 0.001063492	y = (0.034782381)x + 0.001124603
Slope (m)	0.030895714	0.034782381
Intercept (c)	0.001063492	0.001124603
LOD ($\mu g\ mL^{-1}$)	0.218077361	0.129641597
LOQ ($\mu g\ mL^{-1}$)	0.660840488	0.392853325
Standard error	0.000264883	0.000233506

*Mean of six observations

TABLE-17
QUANTIFICATION OF FORMULATION
[SYNASMA-AX]
(ABSORBANCE RATIO 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	398.74	99.67	99.57	0.23814	0.05979	0.09722
	2	400	398.08	99.52				
	3	400	398.36	99.59				
	4	400	398.14	99.54				
	5	400	398.20	99.55				
	6	400	398.25	99.56				
AMB	1	30	29.71	99.03	98.88	0.15579	0.52331	0.0636
	2	30	29.47	98.23				
	3	30	29.40	98.00				
	4	30	29.62	98.73				
	5	30	29.67	99.90				
	6	30	29.82	99.40				

*****Mean of six observations

TABLE-18
INTRADAY AND INTERDAY ANALYSIS OF FORMULATION
[SYNASMA-AX]
(ABSORBANCE RATIO METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.	
			Intraday	Interday	Intraday	Interday	Intraday	Interday
DOX	1	400	99.62	99.69	0.53349	0.48811	0.13374	0.12245
	2	400	99.70	99.52				
	3	400	99.62	99.57				
	4	400	99.90	99.74				
	5	400	99.69	99.54				
	6	400	99.90	99.65				
Mean			99.74	99.62				
AMB	1	30	98.93	99.43	0.20973	0.19799	0.70482	0.66480
	2	30	99.28	99.03				
	3	30	99.91	99.17				
	4	30	99.15	98.70				
	5	30	98.70	99.65				
	6	30	99.16	99.55				
Mean			99.19	99.26				

*Mean of three observations

TABLE-19
RUGGEDNESS STUDY OF FORMULATION
[SYNASMA-AX]
(ABSORBANCE RATIO METHOD)

Drug	Condition	Average* % Obtained	S.D	% R.S.D	S.E.
DOX	Analyst 1	99.61	0.43500	0.10918	0.17759
	Analyst 2	99.67	0.42239	0.10595	0.17244
	Instrument 1	99.61	0.43349	0.10879	0.17697
	Instrument 2	99.65	0.44678	0.11213	0.18239
AMB	Analyst 1	99.72	0.20096	0.67175	0.08204
	Analyst 2	99.41	0.22669	0.76009	0.09254
	Instrument 1	99.43	0.23091	0.77409	0.09427
	Instrument 2	99.51	0.24123	0.81303	0.09848

*****Mean of six observations

TABLE-20
RECOVERY STUDY DATA OF 50% PRE-ANALYSED FORMULATION
[SYNASMA-AX]
(ABSORBANCE RATIO METHOD)

Drug	Perce -ntage	Amount present* ($\mu g\ ml^{-1}$)	Amount added* ($\mu g\ ml^{-1}$)	Amount estimated* ($\mu g\ ml^{-1}$)	Amount recovered* ($\mu g\ ml^{-1}$)	% Recovery*	S.D.	% R.S.D.	S.E.
DOX	80	13.9429	11.2	25.19887	11.25597	100.50	0.013979	0.12419	0.00807
	100	13.9429	14	27.98503	14.04213	100.30	0.004102	0.02921	0.002368
	120	13.9429	16.8	30.82167	16.87877	100.47	0.017989	0.106578	0.10385
AMB	80	1.0367	0.8	1.82873	0.79203	99.00	0.00665	0.83999	0.00384
	100	1.0367	1.0	2.034267	0.99757	99.76	0.00648	0.64998	0.00374
	120	1.0367	1.2	2.234633	1.19793	99.83	0.01019	0.85072	0.00588

*Mean of three observations

TABLE -21
SYSTEM SUITABILITY PARAMETERS FOR THE OPTIMIZED CHROMATOGRAM OF DOXOFYLLINE AND AMBROXOL HYDROCHLORIDE FOR RP-HPLC METHOD

PARAMETERS	DOXOFYLLINE	AMBROXOL HYDROCHLORIDE
Tailing factor	1.55	1.44
Asymmetrical factor	1.67	1.62
Theoretical plates	3951	4519
Capacity factor	1.87	3.48

TABLE-22
STABILITY STUDY OF DOXOFYLLINE AND AMBROXOL HYDROCHLORIDE FOR HPLC METHOD

DRUG: Doxofylline + Ambroxol Hydrochloride
Mobile phase: Phosphate buffer: Acetonitrile: Methanol
Ratio: 70: 20: 10

S.No	Time	Doxofylline at 274 nm	Ambroxol Hydrochloride at 244.5 nm
1	0 min	0.354	0.263
2	10 min	0.353	0.264
3	20 min	0.350	0.263
4	30 min	0.355	0.261
5	40 min	0.353	0.262
6	50 min	0.352	0.263
7	1 hour	0.353	0.264
8	1 hour 15 min	0.352	0.260
9	1 hour 30 min	0.353	0.261
10	1 hour 45 min	0.351	0.262
11	2 hours	0.354	0.264
12	2 hours 30 min	0.352	0.265
13	3 hours	0.350	0.264
14	3 hours 30 min	0.351	0.267
15	4 hours	0.355	0.271
16	5 hours	0.354	0.273

TABLE-23
OPTICAL CHARACTERISTICS OF DOXOFYLLINE AND AMBROXOL HYDROCHLORIDE IN RP-HPLC METHOD

PARAMETERS	DOXOFYLLINE*	AMBROXOL HYDROCHLORIDE*
λ_{max} (nm)	274 nm	244.5 nm
Beers law limit ($\mu g\ mL^{-1}$)	7 -35	0.5 – 2.5
Correlation coefficient (r^2)	0.999707379	0.999765561
Régression equation (y = mx + c)	y = 169886.3316 x + (-17753.96429)	y = 270225.0286 x + (-3.70062 E-09)
Slope (m)	169886.3316	270225.0286
Intercept (c)	-17753.96429	-3.70062 E-09
Standard Error	68094.73019	6120.552086

*Mean of three observations

TABLE -24
QUANTIFICATION OF FORMULATION
[SYNASMA-AX]
BY RP-HPLC

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)	Percentage Obtained*	Average (%)	S.D.	% R.S.D.	S.E.
DOX	1	400	404.2	101.05	100.82	1.92215	0.47582	0.78472
	2		405.6	101.40				
	3		403.3	100.82				
	4		400.5	100.12				
	5		404.5	101.12				
	6		405.7	101.42				
AMB	1	30	30.14	100.46	100.12	0.51887	1.71792	0.21183
	2		30.15	100.50				
	3		30.16	100.53				
	4		29.64	98.80				
	5		29.88	99.60				
	6		30.25	100.83				

* Mean of six observations

TABLE-25
RECOVERY STUDY DATA OF 50% PREANALYSED FORMULATION [SYNASMA-AX] BY RP-HPLC

Drug	Perce -ntage	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.
	80	14.1671	11.2	24.8706	11.0368	98.54	0.03429	0.31075	0.01980
DOX	100	14.1671	14	28.0212	13.8541	98.96	0.00035	0.00249	0.00019
	120	14.1671	16.8	30.8721	16.70503	99.43	5.7735E-05	0.00035	0.00003
	80	1.0533	0.8	1.8401	0.7869	98.36	0.00173	0.22010	0.00099
AMB	100	1.0533	1.0	2.04017	0.98687	98.36	0.00214	0.21644	0.00123
	120	1.0533	1.2	2.25049	1.1972	99.76	0	0	0

*Mean of three observations

TABLE-26
INTRADAY AND INTERDAY ANALYSIS OF FORMULATION [SYNASMA-AX] BY RP-HPLC METHOD

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.	
			Intraday	Interday	Intraday	Interday	Intraday	Interday
	1	400	100.70	100.50				
DOX	2	400	100.79	97.64	0.50817	0.46576	1.69804	1.57263
	3	400	97.80	98.03				
Mean			99.76	98.72				
	1	30	99.12	99.69				
AMB	2	30	99.55	98.97	2.77081	1.53285	0.69469	0.62578
	3	30	100.47	99.56				
Mean			99.71	99.41				

*****Mean of three observations

TABLE-27
SOLUBILITY PROFILE OF METOLAZONE AND SPIRONOLACTONE IN POLAR AND NON POLAR SOLVENTS

S.NO	SOLVENTS	METOLAZONE	SPIRONOLACTONE
1	Distilled Water	Practically insoluble	Practically insoluble
2	0.1M Sodium hydroxide	Slightly soluble	Practically insoluble
3	0.1 M Hydrochloric acid	Practically insoluble	Practically insoluble
4	Glacial acetic acid	Very slightly soluble	Sparingly soluble
5	Methanol	Sparingly soluble	Slightly soluble
6	Ethanol	Sparingly soluble	Slightly soluble
7	Isopropyl alcohol	Practically insoluble	Very slightly soluble
8	n-butanol	Very slightly soluble	-
9	Dimethyl formamide	Freely soluble	Freely soluble
10	Acetonitrile	Sparingly soluble	Sparingly soluble
11	Ethyl acetate	Very slightly soluble	Very slightly soluble
12	Acetone	Sparingly soluble	Sparingly soluble
13	Dichloromethane	Practically insoluble	-
14	Chloroform	Practically insoluble	Sparingly soluble
15	Diethyl ether	Practically insoluble	-
16	Carbon tetrachloride	Practically insoluble	-
17	Petroleum ether	Practically insoluble	-
18	Benzene	-	Sparingly soluble

TABLE-28
STABLILITY STUDY OF METOLAZONE AND SPIRONOLACTONE FOR UV SPECTROSCOPIC METHODS

Solvent: Methanol followed by 0.02 M Phosphate buffer pH 3.5 adjusted with orthophosphoric acid
Concentration of Metolazone and Spironolactone: 10 µg/ml

S.No	Time	Absorbance of Metolazone (236.5 nm)	Absorbance of Spironolactone (242.5 nm)
1	0 min	2.290	0.496
2	10 min	2.290	0.497
3	20 min	2.292	0.497
4	30 min	2.291	0.501
5	40 min	2.290	0.496
6	50 min	2.290	0.498
7	60 min	2.294	0.492
8	1 hour 15 min	2.291	0.493
9	1 hour 30 min	2.294	0.491
10	1 hour 45 min	2.290	0.491
11	2 hours	2.288	0.492
12	2 hours 30 min	2.291	0.493
13	3 hours	2.294	0.491
14	3 hours 30 min	2.230	0.494
15	4 hours	2.231	0.498
16	5 hours	2.234	0.499
17	24 hours	2.334	0.514

TABLE-29
OPTICAL CHARACTERISTICS OF METOLAZONE
(SIMULTANEOUS EQUATION METHOD)

PARAMETERS	AT 236.5 nm*	AT 242.5 nm*
Beer's law limit (μg mL^{-1})	0.5-2.5 μg mL^{-1}	0.5-2.5 μg mL^{-1}
Molar absorptivity	46448.87	36809.60
Sandell's sensitivity (μg/cm^2/0.001 A.U)	0.007885	0.0100613
Correlation coefficient (r)	0.9998	0.9995
Regression equation (y= mx + c)	y = (0.1268571)x + 0.0010785	y = 0.1004990 x + 0.00117619
Slope (m)	0.1268571	0.1004990
Intercept (c)	0.0010785	0.00117619
LOD (μg mL^{-1})	0.0489834	0.076456
LOQ (μg mL^{-1})	0.1484345	0.231685
Standard error	0.0003785	0.0004739

*Mean of six observations

TABLE-30
OPTICAL CHARACTERISTICS OF SPIRONOLACTONE
(SIMULTANEOUS EQUATION METHOD)

PARAMETERS	AT 236.5 nm*	AT 242.5 nm*
Beer's law limit (μg mL^{-1})	5-25 μg ml^{-1}	5-25 μg ml^{-1}
Molar absorptivity	16782.83	17749.80
Sandell's sensitivity (μg/cm^2/ 0.001 A.U)	0.024829	0.023654
Correlation coefficient (r)	0.9999	0.9999
Regression equation (y= mx + c)	y = (0.040395)x + (-0.001104)	y = (0.0425457)x + 0.0006063
Slope (m)	0.040395	0.0425457
Intercept (c)	-0.001104	0.0006063
LOD (μg mL^{-1})	0.356395	0.6907166
LOQ (μg mL^{-1})	1.079985	2.093654
Standard error	0.000692	0.0016894

*Mean of six observations

TABLE-31
QUANTIFICATION OF FORMULATION
[METOLACTONE-5]
(SIMULTANEOUS EQUATION METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D.	S.E.
MET	1	5	5.09	101.80	100.37	0.05776	1.15105	0.02358
	2	5	5.00	100.00				
	3	5	5.04	100.80				
	4	5	5.07	101.40				
	5	5	4.95	99.00				
	6	5	4.96	99.20				
SPIR	1	50	50.09	100.18	100.45	0.115007	0.22899	0.046952
	2	50	50.24	100.48				
	3	50	50.16	100.32				
	4	50	50.14	100.28				
	5	50	50.39	100.78				
	6	50	50.32	100.64				

*****Mean of six observations

TABLE-32
INTRADAY AND INTERDAY ANALYSIS OF FORMULATION
[METOLACTONE-5]
(SIMULTANEOUS EQUATION METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.	
			Intraday	Interday	Intraday	Interday	Intraday	Interday
MET	1	5	100.07	100.10	0.052434	0.052902	1.04542	1.05537
	2	5	100.67	100.70				
	3	5	99.93	100.00				
	4	5	100.60	100.00				
	5	5	100.33	100.10				
	6	5	100.20	100.60				
Mean			100.30	100.25				
SPIR	1	50	100.57	100.45	0.108203	0.052901	0.215447	1.05537
	2	50	100.40	100.40				
	3	50	100.58	100.62				
	4	50	100.27	100.50				
	5	50	100.37	100.43				
	6	50	100.47	100.39				
Mean			100.44	100.47				

*Mean of three observations

TABLE-33
RUGGEDNESS STUDY OF FORMULATION
[METOLACTONE-5]
(SIMULTANEOUS EQUATION METHOD)

Drug	Condition	Average* % Obtained	S.D	% R.S.D	S.E.
MET	Analyst 1	100.33	0.04227	0.84257	0.01726
	Analyst 2	100.73	0.04457	0.88495	0.01819
	Instrument 1	101.00	0.04231	0.83696	0.01727
	Instrument 2	101.09	0.04456	0.87376	0.01819
SPIR	Analyst 1	100.62	0.10944	0.21753	0.04468
	Analyst 2	100.44	0.08329	0.16584	0.03400
	Instrument 1	100.71	0.07394	0.14684	0.03019
	Instrument 2	100.36	0.06984	0.13877	0.02851

*****Mean of six observations

TABLE-34
RECOVERY STUDY DATA OF 50% PRE-ANALYSED FORMULATION
[METOLACTONE-5]
(SIMULTANEOUS EQUATION METHOD)

Drug	Perce -ntage	Amount present* (µg ml^{-1})	Amount added* (µg ml^{-1})	Amount estimated* (µg ml^{-1})	Amount recovered* (µg ml^{-1})	% Recovery*	S.D.	% RSD	S.E.
MET	80	1.0053	0.8	1.8027	0.7974	99.67	0.00155	0.19438	0.00089
	100	1.0053	1	2.0048	0.9995	99.96	0.00263	0.26313	0.00152
	120	1.0053	1.2	2.2084	1.2031	100.27	0.00636	0.52863	0.00367
SPIR	80	10.0552	8	18.0083	7.9531	99.41	0.01019	0.12816	0.00588
	100	10.0552	10	20.0872	10.0319	100.32	0.01749	0.17438	0.01010
	120	10.0552	12	22.2187	12.1635	101.36	0.01575	0.12949	0.00909

*Mean of three observations

TABLE-35
OPTICAL CHARACTERISTICS OF METOLAZONE
(ABSORBANCE CORRECTION METHOD)

PARAMETERS	AT 242.5 nm*	AT 345 nm*
Beer's law limit (µg mL^{-1})	0.5-2.5 µg mL^{-1}	0.5-2.5 µg mL^{-1}
Molar absorptivity L $mol^{-1}cm^{-1}$	36809.60	8093.600
Sandell's sensitivity (µg/cm^2/0.001 A.U)	0.0100613	0.045643
Correlation coefficient (r)	0.9995	0.9999
Regression equation (y= mx + c)	y = 0.1004990 x + 0.00117619	y = 0.022137x + (-0.0001380)
Slope (m)	0.1004990	0.022137
Intercept (c)	0.00117619	-0.0001380
LOD (µg mL^{-1})	0.076456	0.0530679
LOQ (µg mL^{-1})	0.231685	0.1608119
Standard error	0.0004739	3.82133E-05

*Mean of six observations

TABLE-36
OPTICAL CHARACTERISTICS OF SPIRONOLACTONE
(ABSORBANCE CORRECTION METHOD)

PARAMETERS	AT 242.5 nm*	AT 345 nm*
Beer's law limit (µg ml^{-1})	5-25 µg mL^{-1}	-
Molar absorptivity	17749.80	-
Sandell's sensitivity (µg/cm^2/0.001 A.U)	0.023654	-
Correlation coefficient (r^2)	0.9999	-
Regression equation (y = mx + c)	y = (0.0425457)x + 0.0006063	-
Slope (m)	0.0425457	-
Intercept (c)	0.0006063	-
LOD (µg mL^{-1})	0.6907166	-
LOQ (µg mL^{-1})	2.093654	-
Standard error	0.0016894	-

*****Mean of six observations

TABLE-37
QUANTIFICATION OF FORMULATION
[METOLACTONE-5]
(ABSORBANCE CORRECTION METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D.	S.E.
MET	1	5	4.99	99.80	100.17	0.04792	0.95688	0.01957
	2	5	5.08	101.60				
	3	5	4.95	99.00				
	4	5	4.97	99.40				
	5	5	5.02	100.40				
	6	5	5.04	100.80				
SPIR	1	50	50.05	100.10	100.06	0.09445	0.18878	0.03856
	2	50	49.88	99.76				
	3	50	50.11	100.22				
	4	50	50.13	100.26				
	5	50	50.05	100.10				
	6	50	49.96	99.92				

*****Mean of six observations

TABLE-38
INTRADAY AND INTERDAY ANALYSIS OF FORMULATION
[METOLACTONE-5]
(ABSORBANCE CORRECTION METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.	
			Intraday	Interday	Intraday	Interday	Intraday	Interday
MET	1	5	100.27	101.30	0.04384	0.04029	0.87397	0.80393
	2	5	100.13	99.90				
	3	5	100.13	99.30				
	4	5	100.00	100.1				
	5	5	100.40	99.80				
	6	5	100.87	100.80				
Mean			100.30	100.20				
SPIR	1	50	100.07	99.84	0.10045	0.10548	0.20075	0.21296
	2	50	100.11	99.70				
	3	50	100.11	100.25				
	4	50	100.19	99.98				
	5	50	100.06	100.00				
	6	50	99.91	99.89				
Mean			100.08	99.94				

*Mean of three observations

TABLE-39
RUGGEDNESS STUDY OF FORMULATION
[METOLACTONE-5]
(ABSORBANCE CORRECTION METHOD)

Drug	Condition	Average* % Obtained	S.D	% R.S.D	S.E.
MET	Analyst 1	100.23	0.03125	0.62358	0.01276
	Analyst 2	100.23	0.03125	0.62358	0.01276
	Instrument 1	100.17	0.02927	0.58440	0.01195
	Instrument 2	100.14	0.02861	0.57106	0.01168
SPIR	Analyst 1	99.69	0.14665	0.29421	0.05987
	Analyst 2	99.72	0.12303	0.24676	0.05023
	Instrument 1	99.84	0.15587	0.31223	0.06363
	Instrument 2	99.58	0.14982	0.30181	0.06116

*****Mean of six observations

TABLE-40
RECOVERY STUDY DATA OF 50% PRE-ANALYSED FORMULATION
[METOLACTONE-5]
(ABSORBANCE CORRECTION METHOD)

Drug	Percentage	Amount present* ($\mu g\ ml^{-1}$)	Amount added* ($\mu g\ ml^{-1}$)	Amount estimated* ($\mu g\ ml^{-1}$)	Amount recovered* ($\mu g\ ml^{-1}$)	% Recovery*	S.D.	% R.S.D.	S.E.
MET	80	1.0030	0.8	1.8026	0.7996	99.95	0.00687	0.85968	0.00397
	100	1.0030	1.0	2.0059	1.0029	100.29	0.00687	0.68541	0.00397
	120	1.0030	1.2	2.2016	1.2001	100.01	0.00259	0.21648	0.00149
SPIR	80	10.0173	8.0	18.06737	8.05007	100.63	0.02311	0.28712	0.01334
	100	10.0173	10.0	20.07083	10.05353	100.53	0.02079	0.20679	0.01060
	120	10.0173	12.0	22.07983	12.06253	100.52	0.01005	0.08329	0.00580

*Mean of three observations

TABLE-41
OPTICAL CHARACTERISTICS OF METOLAZONE AND SPIRONOLACTONE
(FIRST ORDER DERIVATIVE METHOD)

Parameters	**First derivative method**	
	289 nm	266 nm
	Metolazone	**Spironolactone**
Beer's law limit (µg ml^{-1})	1-5	10-50
Molar absorptivity L $mol^{-1}cm^{-1}$	682.4295159	350.1093
Sandell's sensitivity (µg/cm^2/0.001 A.U)	0.5370689	1.2193285
Correlation coefficient (r^2)	0.9998	0.9999
Regression equation (y= mx + c)	y = 0.001846 x + 6.94444E-06	y = 0.0008202 x + 0.0002013
Slope (m)	0.001846	0.0008202
Intercept (c)	6.94444E-06	0.0002013
LOD (µg mL^{-1})	0.0276462	0.4184765
LOQ (µg mL^{-1})	0.0837765	1.2681107
Standard error	7.0918E-05	0.00015477

TABLE-42
QUANTIFICATION OF FORMULATION
[METOLACTONE-5]
(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.**
MET	1	5	4.99	99.80	100.20	0.06387	1.27495	0.02608
	2	5	5.08	101.60				
	3	5	5.00	100.00				
	4	5	4.91	98.20				
	5	5	5.00	100.00				
	6	5	5.08	101.60				
SPIR	1	50	50.16	100.32	99.97	0.15501	0.31009	0.06328
	2	50	49.95	99.90				
	3	50	49.75	99.50				
	4	50	49.95	99.90				
	5	50	49.95	99.90				
	6	50	50.16	100.32				

***Mean of six observations

TABLE-43
INTRADAY AND INTERDAY ANALYSIS OF FORMULATION
[METOLACTONE-5]
(FIRST ORDER DERIVATIVE METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.	
			Intraday	Interday	Intraday	Interday	Intraday	Interday
MET	1	5	101.07	99.10	0.06797	0.06623	1.35431	1.32968
	2	5	99.33	101.70				
	3	5	99.93	100.00				
	4	5	99.87	99.10				
	5	5	100.40	100.90				
	6	5	101.60	99.10				
Mean			100.37	99.98				
SPIR	1	50	99.75	100.20	0.15456	0.29157	0.30948	0.58321
	2	50	99.91	100.00				
	3	50	99.91	99.12				
	4	50	99.75	100.40				
	5	50	99.77	100.40				
	6	50	100.18	100.00				
Mean			99.88	100.02				

*Mean of three observations

TABLE-44
RUGGEDNESS STUDY OF FORMULATION
[METOLACTONE-5]
(FIRST ORDER DERIVATIVE METHOD)

Drug	Condition	Average* % Obtained	S.D	% R.S.D	S.E.
MET	Analyst 1	99.93	0.08066	1.61435	0.03293
	Analyst 2	100.60	0.07348	1.46093	0.30000
	Instrument 1	99.88	0.09124	1.21345	0.03725
	Instrument 2	99.87	0.08761	1.32123	0.03577
SPIR	Analyst 1	100.03	0.19664	0.39317	0.08028
	Analyst 2	99.92	0.14932	0.29887	0.06096
	Instrument 1	99.79	0.08754	0.67843	0.03574
	Instrument 2	99.84	0.07654	0.72357	0.03125

*****Mean of six observations

TABLE-45
RECOVERY STUDY DATA OF 50% PRE-ANALYSED FORMULATION [METOLACTONE-5]
(FIRST ORDER DERIVATIVE METHOD)

Drug	Perce -ntage	Amount present* ($\mu g\ ml^{-1}$)	Amount added* ($\mu g\ ml^{-1}$)	Amount estimated* ($\mu g\ ml^{-1}$)	Amount recovered* ($\mu g\ ml^{-1}$)	% Recovery*	S.D.	% R.S.D.	S.E.
	80	3.0084	2.4	5.43057	2.42217	100.92	0.03095	1.27778	0.01787
MET	100	3.0084	3.0	6.0026	2.9942	99.81	0.0536	1.77009	0.03095
	120	3.0084	3.6	6.61047	3.60207	100.06	0.03095	0.85912	0.01787
	80	30.0092	24.0	54.04633	4.03713	100.16	0.07038	0.29279	0.04063
SPIR	100	30.0092	30.0	60.10133	30.09213	100.31	0.12195	0.40526	0.07041
	120	30.0092	36.0	66.11573	36.10653	100.29	0.07038	0.19492	0.04063

*Mean of three observations

TABLE -46
SYSTEM SUITABILITY PARAMETERS FOR THE OPTIMIZED CHROMATOGRAM OF METOLAZONE AND SPIRONOLACTONE IN RP-HPLC METHOD

PARAMETERS	METOLAZONE	SPIRONOLACTONE
Tailing factor	1.47	1.60
Asymmetrical factor	1.39	1.43
Theoretical plates	2088	2372
Capacity factor	0.91	4.50

TABLE-47
STABILITY STUDY OF METOLAZONE AND SPIRONOLACTONE FOR RP-HPLC METHOD

DRUG: Metolazone + Spironolactone
Mobile phase: Phosphate buffer 25 mM (pH 3.5): Acetonitrile: Methanol
Ratio: 40: 30: 30

S.No	Time	Metolazone at 236.5 nm	Spironolactone at 243 nm
1	0 min	2.241	0.514
2	10 min	2.244	0.516
3	20 min	2.239	0.509
4	30 min	2.240	0.513
5	40 min	2.243	0.510
6	50 min	2.241	0.515
7	1 hour	2.238	0.516
8	1 hour 15 min	2.239	0.511
9	1 hour 30 min	2.242	0.513
10	1 hour 45 min	2.245	0.512
11	2 hours	2.238	0.516
12	2 hours 30 min	2.239	0.508

TABLE-48
OPTICAL CHARACTERISTICS OF METOLAZONE AND SPIRONOLACTONE IN RP-HPLC METHOD

PARAMETERS	METOLAZONE*	SPIRONOLACTONE*
λ_{max} (nm)	236.5 nm	243 nm
Beers law limit ($\mu g\ mL^{-1}$)	0.5 - 2.5	5-25
Correlation coefficient (r^2)	0.99980474	0.999711299
Regression equation ($y = mx + c$)	y = 1745969.295 x + 32452.1587	y = 169886.3316 x + (-17753.96429)
Slope (m)	1745969.295	642839.3238
Intercept (c)	32452.1587	16795.06348
Standard Error	35676.62233	148015.125986
Sandell's sensitivity	5.73231E-10	1.555457E-09
Molar absorptivity	6.39932E + 11	2.68504E + 11

*Mean of three observations

TABLE -49
QUANTIFICATION OF FORMULATION
[METOLACTONE-5]
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.
MET	1	5	4.88	97.60	99.23	0.09968	0.10045	0.04101
	2	5	5.02	100.40				
	3	5	5.09	101.80				
	4	5	4.88	97.60				
	5	5	4.86	97.20				
	6	5	5.04	100.80				
SPIR	1	50	49.89	99.79	100.29	0.19263	0.38414	0.07864
	2	50	49.94	99.88				
	3	50	50.22	100.44				
	4	50	50.35	100.70				
	5	50	50.16	100.32				
	6	50	50.32	100.64				

* Mean of six observations

TABLE-50
RECOVERY STUDY DATA OF 50 % PRE-ANALYSED FORMULATION
[METOLACTONE-5]
BY RP-HPLC

Drug	Percentage	Amount present* ($\mu g\ ml^{-1}$)	Amount added* ($\mu g\ ml^{-1}$)	Amount estimated* ($\mu g\ ml^{-1}$)	Amount recovered* ($\mu g\ ml^{-1}$)	% Recovery*	S.D.	% R.S.D.	S.E.
MET	80	0.9926	0.8	1.7949	0.80227	100.28	0.00782	0.97511	0.00452
	100	0.9926	1.0	1.98997	0.99737	99.74	0.00343	0.34400	0.00198
	120	0.9926	1.2	2.1965	1.2039	100.33	0.00142	0.11778	0.00082
SPIR	80	10.0254	8.0	18.10597	8.08057	101.08	0.00206	0.02549	0.00119
	100	10.0254	10.0	20.07453	10.04913	100.49	0.03365	0.33485	0.01943
	120	10.0254	12.0	22.01713	11.99173	99.93	0.03591	0.29946	0.02073

*Mean of three observations

TABLE-51
INTRADAY AND INTERDAY ANALYSIS OF FORMULATION [METOLACTONE-5] BY RP-HPLC METHOD

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.	
			Intraday	Interday	Intraday	Interday	Intraday	Interday
MET	1	5	100.00	97.20	0.115036	0.127017	0.11512	0.12685
	2	5	97.60	101.60				
	3	5	102.20	101.60				
Mean			99.93	100.13				
SPIR	1	50	99.70	100.54	0.04358	0.11326	0.04368	0.11279
	2	50	99.86	100.38				
	3	50	99.72	100.32				
Mean			99.76	100.41				

* Mean of three observations

TABLE-52
SOLUBILITY PROFILE OF METOPROLOL AND OLMESARTAN IN POLAR AND NON POLAR SOLVENTS

S.NO	SOLVENTS	METOPROLOL	OLMESARTAN
1	Distilled Water	Sparingly soluble	Practically insoluble
2	0.1M Sodium hydroxide	Slightly soluble	Sparingly soluble
3	0.1 M Hydrochloric acid	Slightly soluble	Practically insoluble
4	Methanol	Freely soluble	Sparingly soluble
5	n-butanol	-	Slightly soluble
6	Acetone	Soluble	Soluble
7	Dimethyl formamide	Slightly soluble	Freely soluble
8	Diethyl ether	Practically insoluble	Practically insoluble
9	Petroleum ether	-	Practically insoluble
10	Chloroform	Sparingly soluble	Sparingly soluble
11	Glacial acetic acid	-	Practically insoluble
12	Isopropyl alcohol	-	Slightly soluble
13	Ethyl acetate	Sparingly soluble	Very Slightly soluble
14	Cyclohexane	-	Practically insoluble
15	Benzene	-	Practically insoluble
16	Dichloromethane	-	Sparingly soluble
17	Ethanol	Soluble	Sparingly soluble
18	Toluene	-	Practically insoluble
19	Carbon tetrachloride	-	Practically insoluble
20	Acetonitrile	Sparingly soluble	Sparingly soluble
21	Dichloroethane	Practically insoluble	-

TABLE-53
STABILITY STUDY OF METOPROLOL AND OLMESARTAN FOR UV SPECTROSCOPIC METHODS

Solvent: Methanol

Concentration of Metoprolol Succinate and Olmesartan Medoxomil: 10 µg/ml

S.No	Time	Absorbance of Metoprolol (223.5 nm)	Absorbance of Olmesartan (256.5 nm)
1	0 min	0.402	0.668
2	10 min	0.395	0.662
3	20 min	0.393	0.666
4	30 min	0.398	0.666
5	40 min	0.393	0.663
6	50 min	0.396	0.658
7	60 min	0.398	0.667
8	1 hour 15 min	0.398	0.664
9	1 hour 30 min	0.403	0.662
10	2 hours	0.407	0.668
11	2 hours 30 min	0.394	0.655
12	3 hours	0.395	0.646
13	4 hours	0.391	0.642
14	5 hours	0.394	0.644
15	24 hours	0.401	0.638

TABLE-54
OPTICAL CHARACTERISTICS OF METOPROLOL (SIMULTANEOUS EQUATION METHOD)

PARAMETERS	**AT 223.5 nm***	**AT 256.5 nm***
Beer's law limit ($\mu g\ mL^{-1}$)	5-25 ($\mu g\ ml^{-1}$)	5-25 ($\mu g\ ml^{-1}$)
Molar absorptivity	25182.62004	1154.357638
Sandell's sensitivity ($\mu g/cm^2$/0.001 A.U)	0.026435524	0.582921265
Correlation coefficient (r^2)	0.999938149	0.99939175
Regression equation (y= mx + c)	y = 0.03853768 x + 0.001426984	y = 0.001722286 x + 0.000460317
Slope (m)	0.03853768	0.001722286
Intercept (c)	0.001426984	0.000460317
LOD ($\mu g\ mL^{-1}$)	0.330893464	0.649585484
LOQ ($\mu g\ mL^{-1}$)	1.002707467	1.968440862
Standard error	0.000447128	7.92964E-05

*Mean of six observations

TABLE-55
OPTICAL CHARACTERISTICS OF OLMESARTAN
(SIMULTANEOUS EQUATION METHOD)

PARAMETERS	AT 223.5 nm*	AT 256.5 nm*
Beer's law limit (µg mL^{-1})	4-20 (µg mL^{-1})	4-20 (µg mL^{-1})
Molar absorptivity	30253.88042	31084.79576
Sandell's sensitivity (µg/cm^2/ 0.001 A.U)	0.018325376	0.018083059
Correlation coefficient (r^2)	0.999781638	0.999632767
Regression equation (y= mx + c)	y = 0.05460381 x + (0.00423254)	y = 0.05539869 x + 0.002699206
Slope (m)	0.05460381	0.05539869
Intercept (c)	0.00423254	0.002699206
LOD (µg mL^{-1})	0.151352883	0.511782663
LOQ (µg mL^{-1})	0.4586451	1.550856556
Standard error	0.001152518	0.001829894

*****Mean of six observations

TABLE-56
QUANTIFICATION OF FORMULATION
[OLSAR-M 25]
(SIMULTANEOUS EQUATION METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D.	S.E.
METO	1	25	24.97	99.89	100.095	0.04546	0.18167	0.01856
	2	25	25.01	100.04				
	3	25	24.99	99.96				
	4	25	25.03	100.12				
	5	25	25.10	100.40				
	6	25	25.04	100.16				
OLME	1	20	19.89	99.45	99.303	0.02	0.10070	0.00817
	2	20	19.87	99.35				
	3	20	19.86	99.30				
	4	20	19.85	99.27				
	5	20	19.83	99.15				
	6	20	19.86	99.30				

*****Mean of six observations

TABLE-57
QUANTIFICATION OF FORMULATION
[OLMESAR-M]
(SIMULTANEOUS EQUATION METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D.	S.E.
METO	1	25	24.80	99.20	100.20	0.13565	0.54152	0.05539
	2	25	25.12	100.48				
	3	25	25.03	100.12				
	4	25	25.06	100.24				
	5	25	25.20	100.80				
	6	25	25.09	100.36				
OLME	1	20	19.91	99.55	99.33	0.07092	0.35692	0.02895
	2	20	19.97	99.85				
	3	20	19.81	99.05				
	4	20	19.83	99.15				
	5	20	19.89	99.45				
	6	20	19.78	98.90				

*****Mean of six observations

TABLE-58
QUANTIFICATION OF FORMULATION
[OLMAX-M]
(SIMULTANEOUS EQUATION METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D.	S.E.
METO	1	50	50.03	100.06	100.28	0.18099	0.36097	0.07389
	2	50	50.15	100.30				
	3	50	49.89	99.78				
	4	50	50.43	100.86				
	5	50	50.13	100.26				
	6	50	50.21	100.42				
OLME	1	20	19.97	99.85	99.60	0.12806	0.64287	0.05228
	2	20	19.81	99.05				
	3	20	20.10	100.50				
	4	20	19.79	98.95				
	5	20	19.83	99.15				
	6	20	20.02	100.10				

*****Mean of six observations

TABLE-59
INTRADAY AND INTERDAY ANALYSIS OF FORMULATION
[OLSAR-M 25]
(SIMULTANEOUS EQUATION METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.	
			Intraday	Interday	Intraday	Interday	Intraday	Interday
METO	1	25	99.98	100.16	0.02564	0.03968	0.10261	0.15865
	2	25	99.94	100.10				
	3	25	99.91	100.00				
	4	25	99.97	100.18				
	5	25	99.99	99.88				
	6	25	99.91	99.92				
Mean			99.95	100.04				
OLME	1	20	99.31	99.30	0.01479	0.01292	0.07444	0.06500
	2	20	99.39	99.28				
	3	20	99.44	99.35				
	4	20	99.32	99.40				
	5	20	99.40	99.45				
	6	20	99.42	99.35				
Mean			99.38	99.36				

*Mean of three observations

TABLE-60
INTRADAY AND INTERDAY ANALYSIS OF FORMULATION
[OLMESAR-M]
(SIMULTANEOUS EQUATION METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.	
			Intraday	Interday	Intraday	Interday	Intraday	Interday
METO	1	25	99.84	99.64	0.09725	0.04733	0.38916	0.18969
	2	25	100.32	99.56				
	3	25	99.92	100.08				
	4	25	100.56	99.92				
	5	25	99.68	99.76				
	6	25	99.84	99.84				
Mean			100.03	99.80				
OLME	1	20	99.10	99.05	0.03189	0.04719	0.16057	0.80851
	2	20	99.25	99.15				
	3	20	99.45	99.55				
	4	20	99.50	99.25				
	5	20	99.15	99.10				
	6	20	99.30	99.60				
Mean			99.29	99.28				

*Mean of three observations

TABLE-61
INTRADAY AND INTERDAY ANALYSIS OF FORMULATION
[OLMAX-M]
(SIMULTANEOUS EQUATION METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.	
			Intraday	Interday	Intraday	Interday	Intraday	Interday
METO	1	50	100.16	100.02	0.06812	0.03266	0.13605	0.06523
	2	50	100.12	100.10				
	3	50	100.40	100.14				
	4	50	100.02	100.16				
	5	50	100.06	100.20				
	6	50	100.08	100.18				
Mean			100.14	100.13				
OLME	1	20	99.00	99.45	0.03578	0.03656	0.18043	0.18427
	2	20	99.10	99.30				
	3	20	99.40	99.20				
	4	20	99.35	99.05				
	5	20	99.05	99.00				
	6	20	99.00	99.15				
Mean			99.15	99.19				

*Mean of three observations

TABLE-62
RUGGEDNESS STUDY OF FORMULATION
[OLSAR-M 25]
(SIMULTANEOUS EQUATION METHOD)

Drug	Condition	Average* % obtained	S.D	%R.S.D	S.E.
METO	Analyst 1	100.01	0.03312	0.13246	0.01352
	Analyst 2	100.05	0.02658	0.10628	0.01085
	Instrument 1	99.52	0.03847	0.15462	0.01571
	Instrument 2	100.09	0.04521	0.18200	0.01847
OLME	Analyst 1	99.31	0.01169	0.05886	0.00477
	Analyst 2	99.39	0.02229	0.11211	0.00909
	Instrument 1	99.37	0.01033	0.05197	0.00422
	Instrument 2	99.30	0.01988	0.10000	0.00812

*****Mean of six observations

TABLE-63
RUGGEDNESS STUDY OF FORMULATION
[OLMESAR-M]
(SIMULTANEOUS EQUATION METHOD)

Drug	Condition	Average* % obtained	S.D	%R.S.D	S.E.
METO	Analyst 1	99.92	0.05215	0.20876	0.02129
	Analyst 2	100.05	0.06646	0.26573	0.02713
	Instrument 1	99.53	0.04750	0.19092	0.01939
	Instrument 2	99.50	0.03937	0.15824	0.01607
OLME	Analyst 1	99.28	0.02066	0.10403	0.00843
	Analyst 2	99.32	0.03559	0.17920	0.01453
	Instrument 1	99.30	0.01789	0.09008	0.00730
	Instrument 2	99.35	0.01414	0.07116	0.00577

*****Mean of six observations

TABLE-64
RUGGEDNESS STUDY OF FORMULATION
[OLMAX-M]
(SIMULTANEOUS EQUATION METHOD)

Drug	Condition	Average* % obtained	S.D	%R.S.D	S.E.
METO	Analyst 1	100.02	0.06653	0.13301	0.02716
	Analyst 2	100.08	0.05492	0.10975	0.02242
	Instrument 1	99.69	0.09883	0.19727	0.04014
	Instrument 2	99.77	0.02582	0.05176	0.01054
OLME	Analyst 1	99.29	0.01835	0.09239	0.00749
	Analyst 2	99.35	0.02757	0.13875	0.01126
	Instrument 1	99.40	0.01414	0.07113	0.00577
	Instrument 2	99.39	0.01472	0.07404	0.00600

*****Mean of six observations

TABLE-65
RECOVERY STUDY DATA OF 50% PRE-ANALYSED FORMULATION
[OLSAR-M 25]
(SIMULTANEOUS EQUATION METHOD)

Drug	Perce-ntage	Amount present* (µg ml^{-1})	Amount added* (µg ml^{-1})	Amount estimated* (µg ml^{-1})	Amount recovered* (µg ml^{-1})	% Recovery*	S.D.	% RSD	S.E.
METO	80	10.0127	8.0	18.01283	8.00013	100.00	0.00971	0.12139	0.00561
	100	10.0127	10.0	20.01057	9.99787	99.98	0.01916	0.19160	0.110620
	120	10.0127	12.0	22.07083	12.05813	100.48	0.00725	0.06009	0.00418
OLME	80	7.9455	6.4	14.36413	6.41863	100.29	0.00748	0.11654	0.00432
	100	7.9455	8.0	16.03207	8.08657	101.08	0.00687	0.08496	0.00397
	120	7.9455	9.6	17.63607	9.69057	100.94	0.00446	0.04602	0.00257

*Mean of three observations

TABLE-66
RECOVERY STUDY DATA OF 50% PRE-ANALYSED FORMULATION
[OLMESAR-M]
(SIMULTANEOUS EQUATION METHOD)

Drug	Perce-ntage	Amount present* (µg ml^{-1})	Amount added* (µg ml^{-1})	Amount estimated* (µg ml^{-1})	Amount recovered* (µg ml^{-1})	% Recovery*	S.D.	% RSD	S.E.
METO	80	10.0144	8.0	18.0070	7.9926	99.91	0.00021	0.00263	0.00012
	100	10.0144	10.0	19.9730	9.9586	99.59	0.00768	0.07712	0.00443
	120	10.0144	12.0	22.0756	12.0616	100.51	0.00508	0.04212	0.00293
OLME	80	7.9570	6.4	14.3810	6.4213	100.34	0.00569	0.08874	0.00328
	100	7.9570	8.0	16.0553	8.0983	101.23	0.00687	0.08483	0.00396
	120	7.9570	9.6	17.6647	9.7076	101.12	0.00549	0.05655	0.00317

*Mean of three observations

TABLE-67
RECOVERY STUDY DATA OF 50% PRE-ANALYSED FORMULATION [OLMAX-M]
(SIMULTANEOUS EQUATION METHOD)

Drug	Percentage	Amount present* ($\mu g\ ml^{-1}$)	Amount added* ($\mu g\ ml^{-1}$)	Amount estimated* ($\mu g\ ml^{-1}$)	Amount recovered* ($\mu g\ ml^{-1}$)	% Recovery*	S.D.	% RSD	S.E.
METO	80	20.1431	16	36.1555	16.011	100.07	0.00245	0.01530	0.00141
	100	20.1431	20	40.1745	20.0314	100.16	0.00638	0.03185	0.00368
	120	20.1431	24	44.1779	24.0348	100.15	0.00349	0.01452	0.00201
OLME	80	7.9455	6.4	14.3503	6.4057	100.08	0.016063	0.25076	0.00927
	100	7.9455	8.0	16.0552	8.104	101.3	0.01578	0.19472	0.00911
	120	7.9455	9.6	17.5231	9.5718	99.70	0.018874	0.19718	0.01089

*Mean of three observations

TABLE-68
OPTICAL CHARACTERISTICS OF METOPROLOL
(AREA UNDER THE CURVE METHOD)

PARAMETERS	218-228 nm*	246-266 nm*
Beer's law limit ($\mu g\ mL^{-1}$)	5 -25 $\mu g\ mL^{-1}$	5 -25 $\mu g\ mL^{-1}$
Molar absorptivity	233824.6705	24588.03322
Sandell's sensitivity ($\mu g/cm^2$/0.001 A.U)	0.002840979	0.027978919
Correlation coefficient (r^2)	0.999928799	0.999591012
Regression equation (y = mx + c)	y = (0.357921905) x + (0.002653968)	y = 0.039196667 x + (-0.020096667)
Slope (m)	0.357921905	0.039196667
Intercept (c)	0.002653968	-0.020096667
LOD ($\mu g\ mL^{-1}$)	0.352203106	7.351173359
LOQ ($\mu g\ mL^{-1}$)	1.067282139	22.2762829
Standard error	0.003405261	0.002540309

*Mean of six observations

TABLE-69
OPTICAL CHARACTERISTICS OF OLMESARTAN
(AREA UNDER THE CURVE METHOD)

PARAMETERS	218-228 nm*	246-266 nm*
Beer's law limit ($\mu g\ ml^{-1}$)	4-20	4-20
Molar absorptivity	317316.9057	553615.4553
Sandell's sensitivity ($\mu g/cm^2$/0.001 A.U)	0.00174521	0.00103584
Correlation coefficient (r^2)	0.99969673	0.999787103
Regression equation (y = mx + c)	y = (0.573476667)x + (-0.052055556)	y = 0.990764048 x + 0.006853968
Slope (m)	0.573476667	0.990764048
Intercept (c)	-0.052055556	0.006853968
LOD ($\mu g\ mL^{-1}$)	0.185925832	0.273632397
LOQ ($\mu g\ mL^{-1}$)	0.563411612	0.829189083
Standard error	0.015597768	0.02077167

*Mean of six observations

TABLE-70
QUANTIFICATION OF FORMULATION
[OLSAR-M 25]
(AREA UNDER THE CURVE METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D.	S.E.
METO	1	25	24.93	99.72	100.07	0.04535	0.18127	0.01851
	2	25	25.01	100.04				
	3	25	25.05	100.20				
	4	25	25.04	100.16				
	5	25	25.04	100.16				
	6	25	25.04	100.16				
OLME	1	20	19.97	99.85	99.87	0.00516	0.02585	0.00211
	2	20	19.97	99.85				
	3	20	19.98	99.90				
	4	20	19.98	99.90				
	5	20	19.97	99.85				
	6	20	19.97	99.85				

***Mean of six observations

TABLE-71
QUANTIFICATION OF FORMULATION
[OLMESAR-M]
(AREA UNDER THE CURVE METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D.	S.E.
METO	1	25	24.92	99.68	100.09	0.05955	0.23800	0.02431
	2	25	24.99	99.96				
	3	25	25.03	100.12				
	4	25	25.06	100.24				
	5	25	25.08	100.32				
	6	25	25.06	100.24				
OLME	1	20	19.93	99.65	99.77	0.01366	0.06847	0.00558
	2	20	19.95	99.75				
	3	20	19.97	99.85				
	4	20	19.96	99.80				
	5	20	19.96	99.80				
	6	20	19.95	99.75				

*****Mean of six observations

TABLE-72
QUANTIFICATION OF FORMULATION
[OLMAX-M]
(AREA UNDER THE CURVE METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D.	S.E.
METO	1	50	49.86	99.72	100.11	0.10689	0.21357	0.04364
	2	50	50.03	100.06				
	3	50	50.10	100.20				
	4	50	50.08	100.16				
	5	50	50.07	100.14				
	6	50	50.18	100.36				
OLME	1	20	19.98	99.90	99.83	0.01966	0.09845	0803
	2	20	19.99	99.95				
	3	20	19.98	99.90				
	4	20	19.96	99.80				
	5	20	19.94	99.70				
	6	20	19.95	99.75				

*****Mean of six observations

TABLE-73
INTRADAY AND INTERDAY ANALYSIS OF FORMULATION
[OLSAR-M 25]
(AREA UNDER THE CURVE METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.	
			Intraday	Interday	Intraday	Interday	Intraday	Interday
METO	1	25	99.69	100.10	0.04639	0.12934	0.18574	0.51806
	2	25	99.91	99.74				
	3	25	100.12	99.80				
	4	25	99.92	99.76				
	5	25	99.88	100.18				
	6	25	99.85	99.64				
Mean			99.895	99.87				
OLME	1	20	99.90	99.83	0.01176	0.07493	0.05886	0.37368
	2	20	99.88	100.28				
	3	20	99.85	100.38				
	4	20	99.88	99.55				
	5	20	99.95	100.23				
	6	20	99.92	100.45				
Mean			99.896	100.12				

*Mean of three observations

TABLE-74
INTRADAY AND INTERDAY ANALYSIS OF FORMULATION
[OLMESAR-M]
(AREA UNDER THE CURVE METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.	
			Intraday	Interday	Intraday	Interday	Intraday	Interday
METO	1	25	99.68	100.08	0.09109	0.14024	0.36490	0.56088
	2	25	99.56	100.12				
	3	25	100.44	100.96				
	4	25	100.16	99.40				
	5	25	99.60	99.48				
	6	25	99.64	100.04				
Mean			99.85	100.01				
OLME	1	20	99.90	100.00	0.06494	0.05577	0.32437	0.27801
	2	20	100.00	99.90				
	3	20	99.85	100.25				
	4	20	99.80	100.40				
	5	20	100.55	100.60				
	6	20	100.45	100.50				
Mean			100.09	100.28				

*Mean of three observations

TABLE-75
INTRADAY AND INTERDAY ANALYSIS OF FORMULATION
[OLMAX-M]
(AREA UNDER THE CURVE METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.	
			Intraday	Interday	Intraday	Interday	Intraday	Interday
METO	1	50	99.60	99.62	0.07992	0.07155	0.16013	0.14347
	2	50	100.00	99.64				
	3	50	99.96	99.72				
	4	50	99.66	100.00				
	5	50	99.80	99.66				
	6	50	99.86	99.80				
Mean			99.81	99.74				
OLME	1	20	99.90	99.80	0.04604	0.03764	0.23089	0.18858
	2	20	100.00	100.00				
	3	20	99.80	99.85				
	4	20	99.55	99.90				
	5	20	99.45	99.75				
	6	20	99.50	99.45				
Mean			99.70	99.79				

*Mean of three observations

TABLE-76
RUGGEDNESS STUDY OF FORMULATION
[OLSAR-M 25]
(AREA UNDER THE CURVE METHOD)

Drug	Condition	Average* % Obtained	S.D	% R.S.D	S.E.
METO	Analyst 1	99.85	0.05037	0.20177	0.02056
	Analyst 2	99.91	0.09395	0.37615	0.03836
	Instrument 1	99.16	0.03867	0.1540	0.01579
	Instrument 2	100.07	0.04534	0.1820	0.01851
OLME	Analyst 1	99.94	0.00753	0.03766	0.00307
	Analyst 2	99.93	0.01378	0.06897	0.00563
	Instrument 1	99.37	0.01045	0.0520	0.00427
	Instrument 2	99.30	0.01998	0.1000	0.00816

*****Mean of six observations

TABLE-77
RUGGEDNESS STUDY OF FORMULATION
[OLMESAR-M]
(AREA UNDER THE CURVE METHOD)

Drug	Condition	Average* % Obtained	S.D	% R.S.D	S.E.
METO	Analyst 1	99.92	0.07720	0.30905	0.03152
	Analyst 2	99.99	0.03430	0.13725	0.01400
	Instrument 1	99.80	0.07376	0.29563	0.03011
	Instrument 2	99.76	0.07376	0.29563	0.03011
OLME	Analyst 1	99.86	0.04324	0.21663	0.01765
	Analyst 2	99.85	0.04049	0.20275	0.01654
	Instrument 1	99.87	0.02160	0.10817	0.00882
	Instrument 2	99.96	0.02483	0.12421	0.01014

*****Mean of six observations

TABLE-78
RUGGEDNESS STUDY OF FORMULATION
[OLMAX-M]
(AREA UNDER THE CURVE METHOD)

Drug	Condition	Average* % Obtained	S.D	% R.S.D	S.E.
METO	Analyst 1	100.40	0.08128	0.00163	0.03318
	Analyst 2	100.02	0.04591	0.09180	0.01874
	Instrument 1	99.96	0.05719	0.11443	0.02335
	Instrument 2	100.03	0.06321	0.12634	0.02581
OLME	Analyst 1	99.90	0.05324	0.26647	0.02174
	Analyst 2	99.60	0.06120	0.30723	0.02498
	Instrument 1	99.80	0.07318	0.36663	0.02988
	Instrument 2	99.90	0.07425	0.37162	0.03031

*****Mean of six observations

TABLE-79
RECOVERY STUDY DATA OF 50% PRE-ANALYSED FORMULATION
[OLSAR-M 25]
(AREA UNDER THE CURVE METHOD)

Drug	Perce -ntage	Amount present* ($\mu g\ ml^{-1}$)	Amount added* ($\mu g\ ml^{-1}$)	Amount estimated* ($\mu g\ ml^{-1}$)	Amount recovered* ($\mu g\ ml^{-1}$)	% Recovery*	S.D.	% R.S.D.	S.E.
	80	10.0140	8.0	18.08753	8.07353	100.92	0.00412	0.05103	0.00238
METO	100	10.0140	10.0	20.01337	9.99937	99.99	0.03926	0.39262	0.02267
	120	10.0140	12.0	21.98723	11.97323	99.78	0.00454	0.03792	0.00262
	80	7.9948	6.4	14.3918	6.397	99.96	0.00601	0.09395	0.00347
OLME	100	7.9948	8.0	15.98593	7.99113	99.89	0.00404	0.05056	0.00233
	120	7.9948	9.6	17.61163	9.61683	100.17	0.00299	0.03109	0.00173

*Mean of three observations

TABLE-80
RECOVERY STUDY DATA OF 50% PRE-ANALYSED FORMULATION
[OLMESAR-M]
(AREA UNDER THE CURVE METHOD)

Drug	Perce -ntage	Amount present* ($\mu g\ ml^{-1}$)	Amount added* ($\mu g\ ml^{-1}$)	Amount estimated* ($\mu g\ ml^{-1}$)	Amount recovered* ($\mu g\ ml^{-1}$)	% Recovery*	S.D.	% R.S.D.	S.E.
	80	10.0172	8.0	18.0135	7.9963	99.95	0.04145	0.5184	0.02393
METO	100	10.0172	10.0	20.0543	10.0371	100.37	0.03240	0.32280	0.18706
	120	10.0172	12.0	21.9357	11.9185	99.32	0.005014	0.04203	0.00289
	80	7.9923	6.4	14.3125	6.3202	98.75	0.00589	0.09319	0.00340
OLME	100	7.9923	8.0	15.9912	7.9989	99.98	0.00382	0.04776	0.00221
	120	7.9923	9.6	17.6230	9.6307	100.32	0.00312	0.03239	0.00180

*Mean of three observations

TABLE-81
RECOVERY STUDY DATA OF 50% PRE-ANALYSED FORMULATION [OLMAX-M]
(AREA UNDER THE CURVE METHOD)

Drug	Percentage	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.
METO	80	20.1512	16	36.1762	16.02503	100.15	0.00435	0.02672	0.00251
	100	20.1512	20	40.1755	20.0243	100.12	0.00735	0.03670	0.00424
	120	20.1512	24	44.1818	24.0306	100.12	0.00896	0.03728	0.00517
OLME	80	7.9512	6.4	14.3544	6.4032	100.05	0.00589	0.09198	0.00340
	100	7.9512	8.0	15.9852	8.0157	100.19	0.0133	0.16518	0.00768
	120	7.9512	9.6	17.5705	9.6194	100.20	0.00945	0.09824	0.00545

*Mean of three observations

TABLE-82
OPTICAL CHARACTERISTICS OF METOPROLOL AND OLMESARTAN
(FIRST ORDER DERIVATIVE METHOD)

Parameters	FIRST ORDER DERIVATIVE METHOD	
	256 nm	243 nm
	Metoprolol	**Olmesartan**
Beer's law limit(µg ml^{-1})	25-125	20-100
Molar absorptivity L $mol^{-1}cm^{-1}$	85.71989333	318.6634233
Sandell's sensitivity (µg/cm^2/ 0.001 A.U)	7.842581809	1.661527584
Correlation coefficient (r^2)	0.999736206	0.999931462
Regression equation (y= mx + c)	y = 0.000128057 x + 3.25397E-05	y = 0.00060219 x + (-0.000315079)
Slope (m)	0.000128057	0.00060219
Intercept (c)	3.25397E-05	-0.000315079
LOD (µg mL^{-1})	0.24902326	0.083566619
LOQ (µg mL^{-1})	0.754615939	0.253232178
Standard error	0.000148139	0.000271841

TABLE-83
QUANTIFICATION OF FORMULATION
[OLSAR-M 25]
(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.
METO	1	25	24.86	99.44	99.96	0.20139	0.80590	0.082219
	2	25	25.25	101.00				
	3	25	24.86	99.44				
	4	25	24.86	99.44				
	5	25	24.86	99.44				
	6	25	25.25	101.00				
OLME	1	20	20.03	100.15	100.06	0.08377	0.41858	0.03419
	2	20	19.94	99.70				
	3	20	20.11	100.55				
	4	20	19.94	99.70				
	5	20	19.94	99.70				
	6	20	20.11	100.55				

*****Mean of six observations

TABLE-84
QUANTIFICATION OF FORMULATION
[OLMESAR-M]
(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.
METO	1	25	24.90	99.60	99.81	0.07229	0.28974	0.02951
	2	25	25.05	100.20				
	3	25	24.93	99.72				
	4	25	24.91	99.64				
	5	25	24.89	99.56				
	6	25	25.04	100.16				
OLME	1	20	20.01	100.05	100.00	0.05621	0.28105	0.02295
	2	20	19.98	99.90				
	3	20	20.09	100.45				
	4	20	19.98	99.90				
	5	20	19.92	99.60				
	6	20	20.02	100.10				

*****Mean of six observations

TABLE-85
QUANTIFICATION OF FORMULATION
[OLMAX-M]
(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.
	1	50	50.02	100.04				
	2	50	50.32	100.64				
METO	3	50	50.41	100.82	100.51	0.16306	0.3244	0.06657
	4	50	50.12	100.24				
	5	50	50.23	100.46				
	6	50	50.43	100.86				
	1	20	20.12	100.60				
	2	20	19.98	99.90				
	3	20	20.02	100.10	100.15	0.04979	0.2486	0.02032
	4	20	19.99	99.95				
OLME	5	20	20.03	100.15				
	6	20	20.04	100.20				

*****Mean of six observations

TABLE-86
INTRADAY AND INTERDAY ANALYSIS OF FORMULATION
[OLSAR-M 25]
(FIRST ORDER DERIVATIVE METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.	
			Intraday	Interday	Intraday	Interday	Intraday	Interday
	1	25	99.44	99.44				
	2	25	100.47	100.22				
	3	25	100.48	101.00	0.19141	0.18031	0.76436	0.72234
METO	4	25	99.96	99.44				
	5	25	100.48	99.44				
	6	25	99.96	99.44				
Mean			100.13	99.83				
	1	20	99.7	99.50				
	2	20	100.38	99.50				
	3	20	100.10	100.33				
OLME	4	20	99.98	100.33	0.10084	0.12303	0.50339	0.61508
	5	20	100.67	100.10				
	6	20	100.10	100.33				
Mean			100.16	100.06				

*Mean of three observations

TABLE-87
INTRADAY AND INTERDAY ANALYSIS OF FORMULATION
[OLMESAR-M]
(FIRST ORDER DERIVATIVE METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.	
			Intraday	Interday	Intraday	Interday	Intraday	Interday
METO	1	25	99.56	99.32	0.18411	0.18787	0.73556	0.74819
	2	25	101.04	100.92				
	3	25	99.56	101.24				
	4	25	100.08	99.72				
	5	25	101.00	100.72				
	6	25	99.44	100.76				
Mean			100.11	100.45				
OLME	1	20	99.75	99.35	0.12442	0.09445	0.62117	0.47249
	2	20	99.45	99.60				
	3	20	100.15	99.75				
	4	20	100.90	100.05				
	5	20	100.90	100.35				
	6	20	99.75	100.60				
Mean			100.15	99.95				

*Mean of three observations

TABLE-88
INTRADAY AND INTERDAY ANALYSIS OF FORMULATION
[OLMAX-M]
(FIRST ORDER DERIVATIVE METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.	
			Intraday	Interday	Intraday	Interday	Intraday	Interday
METO	1	50	100.62	100.02	0.07251	0.16558	0.14404	0.32905
	2	50	100.84	100.76				
	3	50	100.48	100.60				
	4	50	100.76	100.88				
	5	50	100.82	100.94				
	6	50	100.58	100.66				
Mean			100.68	100.64				
OLME	1	20	100.65	100.60	0.07146	0.09114	0.35659	0.45456
	2	20	100.10	100.20				
	3	20	99.85	100.90				
	4	20	100.05	100.35				
	5	20	100.60	99.90				
	6	20	99.85	99.65				
Mean			100.18	100.27				

*Mean of three observations

TABLE-89
RUGGEDNESS STUDY OF FORMULATION
[OLSAR-M 25]
(FIRST ORDER DERIVATIVE METHOD)

Drug	Condition	Average* % Obtained	S.D	% R.S.D	S.E.
METO	Analyst 1	100.22	0.21361	0.85257	0.08721
	Analyst 2	100.22	0.21361	0.85257	0.08721
	Instrument I	100.12	0.24568	0.87531	0.05945
	Instrument II	100.03	0.32415	0.86756	0.13233
OLME	Analyst 1	100.05	0.12231	0.61125	0.04993
	Analyst 2	99.98	0.13515	0.67588	0.05518
	Instrument I	100.09	0.14563	0.73458	0.05945
	Instrument II	100.01	0.15781	0.76783	0.06443

*Mean of six observations

TABLE-90
RUGGEDNESS STUDY OF FORMULATION
[OLMESAR-M]
(FIRST ORDER DERIVATIVE METHOD)

Drug	Condition	Average* % Obtained	S.D	% R.S.D	S.E.
METO	Analyst 1	100.04	0.08025	0.32087	0.03276
	Analyst 2	100.12	0.079415	0.31728	0.03242
	Instrument I	99.76	0.19959	0.80028	0.08148
	Instrument II	100.00	0.16955	0.67820	0.06922
OLME	Analyst 1	99.65	0.05718	0.28690	0.02334
	Analyst 2	100.10	0.05819	0.2966	0.02375
	Instrument I	99.65	0.01049	0.05263	0.00428
	Instrument II	99.90	0.01033	0.05170	0.00422

*Mean of six observations

TABLE-91
RUGGEDNESS STUDY OF FORMULATION
[OLMAX-M]
(FIRST ORDER DERIVATIVE METHOD)

Drug	Condition	Average* % Obtained	S.D	% R.S.D	S.E.
METO	Analyst 1	100.12	0.03266	0.06524	0.0133
	Analyst 2	100.18	0.02608	0.05207	0.01065
	Instrument I	99.98	0.03312	0.06625	0.01354
	Instrument II	100.12	0.04037	0.08064	0.01648
OLME	Analyst 1	100.10	0.06284	0.31389	0.02565
	Analyst 2	100.05	0.04183	0.2090	0.01708
	Instrument I	99.95	0.05244	0.26233	0.02140
	Instrument II	99.80	0.03869	0.19384	0.01579

*Mean of six observations

TABLE-92
RECOVERY STUDY DATA OF 50% PRE-ANALYSED FORMULATION
[OLSAR-M 25]
(FIRST ORDER DERIVATIVE METHOD)

Drug	Perce -ntage	Amount present* ($\mu g\ ml^{-1}$)	Amount added* ($\mu g\ ml^{-1}$)	Amount estimated* ($\mu g\ ml^{-1}$)	Amount recovered* ($\mu g\ ml^{-1}$)	% Recovery*	S.D.	% R.S.D.	S.E.
METO	80	49.9839	40.0	90.0703	40.0864	100.22	0.45085	1.12470	0.26030
	100	49.9839	50.0	100.2218	50.2379	100.47	0.45068	0.89709	0.26020
	120	49.9839	60.0	110.1133	60.12943	100.21	0.45091	0.74990	0.26033
OLME	80	40.0179	32.0	72.09533	32.07743	100.22	0.16605	0.51765	0.09587
	100	40.0179	40.0	80.01087	39.99297	100.47	0.09584	0.23964	0.05533
	120	40.0179	48.0	88.2032	48.1853	100.21	0.1661	0.34471	0.34471

*Mean of three observations

TABLE-93
RECOVERY STUDY DATA OF 50% PRE-ANALYSED FORMULATION
[OLMESAR-M]
(FIRST ORDER DERIVATIVE METHOD)

Drug	Percentage	Amount present* ($\mu g\ ml^{-1}$)	Amount added* ($\mu g\ ml^{-1}$)	Amount estimated* ($\mu g\ ml^{-1}$)	Amount recovered* ($\mu g\ ml^{-1}$)	% Recovery*	S.D.	% R.S.D.	S.E.
	80	49.7236	40.0	90.0052	40.28163	100.70	0.06034	0.14978	0.03483
METO	100	49.7236	50.0	100.1045	50.3809	100.76	0.10043	0.19934	0.5798
	120	49.7236	60.0	110.1053	60.3817	100.64	0.00610	.01010	0.00352
	80	40.0456	32.0	72.01820	31.9726	99.73	0.019673	0.06153	0.01136
OLME	100	40.0456	40.0	79.9742	39.9286	99.82	0.04909	0.1229	0.02834
	120	40.0456	48.0	88.0087	47.9631	99.92	0.00745	0.01553	0.00430

*Mean of three observations

TABLE-94
RECOVERY STUDY DATA OF 50% PRE-ANALYSED FORMULATION
[OLMAX-M]
(FIRST ORDER DERIVATIVE METHOD)

Drug	Percentage	Amount present* ($\mu g\ ml^{-1}$)	Amount added* ($\mu g\ ml^{-1}$)	Amount estimated* ($\mu g\ ml^{-1}$)	Amount recovered* ($\mu g\ ml^{-1}$)	% Recovery*	S.D.	% R.S.D.	S.E.
	80	100.01234	80.0	180.1689	80.0453	100.05	0.00599	0.00748	0.00346
METO	100	100.02134	100.0	200.1623	100.0389	100.03	0.01902	0.01901	0.01098
	120	100.0213	120.0	220.1715	120.0479	100.04	0.00955	0.00795	0.00551
	80	40.0012	32.0	72.0134	32.0122	100.04	0.1708	0.5335	0.09861
OLME	100	40.0341	40.0	80.0145	39.9804	99.95	0.0835	0.2088	0.04821
	120	40.0156	48.0	88.1341	48.1185	100.25	0.1546	0.3213	0.08926

*Mean of three observations

TABLE-95
SOLUBILITY PROFILE OF ASPIRIN AND ROSUVASTATIN CALCIUM IN POLAR AND NON POLAR SOLVENTS

S.NO	SOLVENTS	ASPIRIN	ROSUVASTATIN CALCIUM
1	Distilled Water	Very Slightly Soluble	Slightly Soluble
2	Methanol	Freely Soluble	Sparingly Soluble
3	Ethanol	Freely Soluble	Sparingly Soluble
4	Acetonitrile	Freely Soluble	Freely Soluble
5	Dichloromethane	Soluble	Freely Soluble
6	Toluene	Slightly Soluble	
7	Benzene	Slightly Soluble	Practically Insoluble
8	Dimethyl formamide	Freely Soluble	Freely Soluble
9	Ethyl acetate	Soluble	
10	n-butanol	Sparingly Soluble	Practically Insoluble
11	Diethyl ether	Sparingly Soluble	Practically Insoluble
12	Chloroform	Soluble	Freely Soluble
13	0.1 M Hcl	Slightly Soluble	Very Slightly Soluble
14	0.1 M NaOH	Sparingly Soluble	Very Slightly Soluble
15	Isopropyl Alcohol	Freely Soluble	Practically Insoluble
16	Hexane	Slightly Soluble	Practically Insoluble
17	Acetic acid	Slightly Soluble	Soluble
18	Acetone	Sparingly Soluble	Freely Soluble
19	Cyclohexane	-	Practically Insoluble
20	Petroleum ether	-	Practically Insoluble
21	Carbon tetrachloride	-	Practically Insoluble

TABLE-96
STABILITY STUDYOF ASPIRIN AND ROSUVASTATIN CALCIUM FOR UV SPECTROSCOPIC METHODS

Solvent: Methanol
Concentration of Aspirin and Rosuvastatin Calcium: 10 μg/ml

S.No	Time	Absorbance of Aspirin (294.5 nm)	Absorbance of Rosuvastatin Calcium (243 nm)
1	0 min	0.040	0.436
2	10 min	0.044	0.432
3	20 min	0.046	0.434
4	30 min	0.045	0.432
5	40 min	0.043	0.435
6	50 min	0.041	0.433
7	60 min	0.044	0.436
8	1 hour 15 min	0.040	0.435
9	1 hour 30 min	0.042	0.432
10	1 hour 45 min	0.043	0.431
11	2 hours	0.045	0.436
12	2 hours 30 min	0.049	0.433
13	3 hours	0.052	0.437
14	3 hours 30 min	0.069	0.452
15	4 hours	0.049	0.469
16	24 hours	0.048	0.471

TABLE-97
OPTICAL CHARACTERISTICS OF ASPIRIN
(SIMULTANEOUS EQUATION METHOD)

PARAMETERS	AT 294.5 nm*	AT 243 nm*
Beer's law limit (µg mL^{-1})	7.5-37.5	1-5
Molar absorptivity	352.6714222	1622.260511
Sandells sensitivity (µg/cm^2/0.001 A.U)	0.513299586	0.111744093
Correlation coefficient (r^2)	0.999825591	0.999935492
Regression equation (y= mx + c)	y= 0.00195219 x + 4.92063E-05	y = 0.008958032 x + 0.000445238
Slope (m)	0.00195219	0.008958032
Intercept (c)	4.92063E-05	0.000445238
LOD (µg mL^{-1})	1.005901606	0.306015714
LOQ (µg mL^{-1})	3.048186685	0.927320345
Standard error	0.000117395	0.000622022

*Mean of six observations

TABLE-98
OPTICAL CHARACTERISTICS OF ROSUVASTATIN CALCIUM
(SIMULTANEOUS EQUATION METHOD)

PARAMETERS	AT 294.5 nm*	AT 243 nm*
Beer's law limit (µg ml^{-1})	1-5	1-5
Molar absorptivity	13869.048721	44206.9075
Sandell's sensitivity (µg/cm^2/ 0.001 A.U)	0.072660601	0.022732742
Correlation coefficient (r^2)	0.999811891	0.999935137
Regression equation (y= mx + c)	y = 0.013849524 x + 4.28571E-05	y = 0.044133333 x + 0.00025
Slope (m)	0.013849524	0.044133333
Intercept (c)	4.28571E-05	0.00025
LOD (µg mL^{-1})	0.137983219	0.075413203
LOQ (µg mL^{-1})	0.418130966	0.228524856
Standard error	9.1004E-05	0.00022363

*Mean of six observations

TABLE-99
QUANTIFICATION OF FORMULATION
[ROZUCOR ASP-10]
(SIMULTANEOUS EQUATION METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D.	S.E.
ASP	1	75	74.91	99.88	99.81	0.08756	0.11697	0.03575
	2		74.85	99.80				
	3		74.91	99.87				
	4		74.96	99.94				
	5		74.73	99.64				
	6		74.78	99.71				
ROSU	1	10	9.99	99.95	99.46	0.02338	0.23497	0.00954
	2		9.94	99.41				
	3		9.92	99.19				
	4		9.94	99.35				
	5		9.94	99.41				
	6		9.95	99.47				

*****Mean of six observations

TABLE-100
INTRADAY AND INTERDAY ANALYSIS OF FORMULATION
[ROZUCOR ASP-10]
(SIMULTANEOUS EQUATION METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.	
			Intraday	Interday	Intraday	Interday	Intraday	Interday
ASP	1	75	99.84	99.85	0.18694	0.07413	0.24849	0.09886
	2		100.12	99.97				
	3		100.24	100.02				
	4		100.44	100.07				
	5		100.11	99.93				
	6		99.94	99.99				
Mean			100.12	99.97				
ROSU	1	10	99.37	99.28	0.03469	0.03071	0.34935	0.30904
	2		99.23	99.29				
	3		99.38	99.69				
	4		99.06	99.38				
	5		99.21	98.08				
	6		99.72	99.36				
Mean			99.33	99.18				

*Mean of three observations

TABLE-101
RUGGEDNESS STUDY OF FORMULATION
[ROZUCOR ASP-10]
(SIMULTANEOUS EQUATION METHOD)

Drug	Condition	Average* % Obtained	S.D	% R.S.D	S.E.
ASP	Analyst 1	99.82	0.03011	0.04018	0.01269
	Analyst 2	99.91	0.02944	0.03929	0.01202
	Instrument 1	99.91	0.02509	0.00033	0.01024
	Instrument 2	99.87	0.02671	0.03566	0.01090
ROSU	Analyst 1	99.55	0.00896	0.08995	0.00366
	Analyst 2	99.56	0.00825	0.08286	0.00337
	Instrument 1	99.73	0.02106	0.21159	0.00859
	Instrument 2	99.91	0.02341	1.17161	0.00956

*****Mean of six observations

TABLE-102
RECOVERY STUDY DATA OF 50% PRE-ANALYSED FORMULATION
[ROZUCOR ASP-10]
(SIMULTANEOUS EQUATION METHOD)

Drug	Perce -ntage	Amount present* (µg ml^{-1})	Amount added* (µg ml^{-})	Amount estimated* (µg ml^{-1})	Amount recovered* (µg ml^{-1})	% Recovery*	S.D.	% RSD	S.E.
ASP	80	14.9713	12	26.9878	12.0165	100.13	0.00568	0.04727	0.00328
	100	14.9713	15	29.981	15.0097	100.06	0.01002	0.01001	0.00578
	120	14.9713	18	32.9778	18.0065	100.04	0.00524	0.02910	0.00303
ROSU	80	1.9892	1.6	3.5807	1.5919	99.47	0.00087	0.05465	0.00050
	100	1.9892	2.0	3.9841	1.9949	99.75	0.0015	0.07519	0.00087
	120	1.9892	2.4	4.3842	2.3950	99.79	0.00591	0.24676	0.00341

*Mean of three observations

TABLE-103
OPTICAL CHARACTERISTICS OF ASPIRIN
(ABSORBANCE RATIO METHOD)

PARAMETERS	AT 229.8 nm*	AT 243 nm*
Beer's law limit ($\mu g\ ml^{-1}$)	7.5-37.5	1-5
Molar absorptivity	4870.485644	1622.260511
Sandell's sensitivity ($\mu g/cm^2$/0.001 A.U)	0.036782029	0.111744093
Correlation coefficient (r^2)	0.999935492	0.999935492
Regression equation (y= mx + c)	y = 0.027229333 x + (-0.00201111)	y = 0.008958032 x + 0.000445238
Slope (m)	0.027229333	0.008958032
Intercept (c)	-0.00201111	0.000445238
LOD ($\mu g\ ml^{-1}$)	0.337241848	0.306015714
LOQ ($\mu g\ ml^{-1}$)	1.021944993	0.927320345
Standard error	0.000622022	0.000622022

*Mean of six observations

TABLE-104
OPTICAL CHARACTERISTICS OF ROSUVASTATIN CALCIUM
(ABSORBANCE RATIO METHOD)

PARAMETERS	AT 229.8 nm*	AT 243 nm*
Beer's law limit ($\mu g\ mL^{-1}$)	1-5	1-5
Molar absorptivity	25498.49371	44206.9075
Sandell's sensitivity ($\mu g/cm^2$/0.001 A.U)	0.039317699	0.022732742
Correlation coefficient (r^2)	0.99989276	0.999935137
Regression equation (y = mx + c)	y = 0.025472857 x + (-2.38095E-05)	y = 0.044133333 x + 0.00025
Slope (m)	0.025472857	0.044133333
Intercept (c)	-2.38095E-05	0.00025
LOD ($\mu g\ mL^{-1}$)	0.036935965	0.075413203
LOQ ($\mu g\ mL^{-1}$)	0.111927167	0.228524856
Standard error	0.000148596	0.00022363

*Mean of six observations

TABLE-105
QUANTIFICATION OF FORMULATION
[ROZUCOR ASP-10]
(ABSORBANCE RATIO METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D.	S.E.
ASP	1	75	74.95	99.93	99.77	0.14516	0.19383	0.05926
	2		74.95	99.93				
	3		74.92	99.89				
	4		74.94	99.92				
	5		74.96	99.47				
	6		74.59	99.45				
ROSU	1	10	9.955	99.55	99.59	0.01099	0.11036	0.00449
	2		9.945	99.45				
	3		9.967	99.67				
	4		9.947	99.47				
	5		9.967	99.67				
	6		9.970	99.70				

*****Mean of six observations

TABLE-106
INTRADAY AND INTERDAY ANALYSIS OF FORMULATION
[ROZUCOR ASP-10]
(ABSORBANCE RATIO METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.	
			Intraday	Interday	Intraday	Interday	Intraday	Interday
ASP	1	75	99.93	99.95	0.01169	0.01862	0.01559	0.01558
	2		99.89	99.88				
	3		99.93	99.92				
	4		99.92	99.95				
	5		99.93	99.92				
	6		99.92	99.93				
Mean			99.92	99.93				
ROSU	1	10	99.45	99.45	0.00169	0.01729	0.01699	0.00173
	2		99.50	99.56				
	3		99.46	99.70				
	4		99.47	99.45				
	5		99.48	99.67				
	6		99.47	99.90				
Mean			99.47	99.62				

*Mean of three observations

TABLE-107
RUGGEDNESS STUDY OF FORMULATION
[ROZUCOR ASP-10]
(ABSORBANCE RATIO METHOD)

Drug	Condition	Average* % Obtained	S.D	% R.S.D	S.E.
ASP	Analyst 1	99.91	0.02338	0.03120	0.00954
	Analyst 2	99.95	0.02451	0.03269	0.01006
	Instrument 1	99.92	0.02613	0.03485	0.01067
	Instrument 2	99.89	0.02468	0.03292	0.01008
ROSU	Analyst 1	99.44	0.01784	0.89702	0.00728
	Analyst 2	99.47	0.01834	0.92365	0.00749
	Instrument 1	99.94	0.01813	0.91146	0.00740
	Instrument 2	99.92	0.01892	0.95105	0.00772

*****Mean of six observations

TABLE-108
RECOVERY STUDY DATA OF 50% PRE-ANALYSED FORMULATION
[ROZUCOR ASP-10]
(ABSORBANCE RATIO METHOD)

Drug	Perce -ntage	Amount present* ($\mu g\ ml^{-1}$)	Amount added* ($\mu g\ ml^{-1}$)	Amount estimated* ($\mu g\ ml^{-1}$)	Amount recovered* ($\mu g\ ml^{-1}$)	% Recovery*	S.D.	% R.S.D.	S.E.
ASP	80	14.9891	12.0	26.9899	12.0008	100.01	0.00125	0.01041	0.00072
	100	14.9891	15.0	29.9734	14.9843	99.89	0.02624	0.17512	0.00072
	120	14.9891	18.0	32.9847	17.9956	99.97	0.00383	0.02129	0.00221
ROSU	80	1.9892	1.6	3.5813	1.5921	99.51	0.00115	0.07223	0.00066
	100	1.9892	2.0	3.9847	1.9955	99.77	0.00165	0.08269	0.00095
	120	1.9892	2.4	4.3855	2.3963	99.85	0.00656	0.27375	0.00379

*Mean of three observations

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Research Article

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC DETERMINATION OF DOXOFYLLINE AND AMBROXOL HYDROCHLORIDE IN BULK AND COMBINED TABLET FORMULATION

B.UMADEVI* AND T. VETRICHELVAN

Department of Pharmaceutical Analysis, Adhiparasakthi College of Pharmacy, Melmaruvathur 603 319 Tamil Nadu, India.
Email: umadevipharm@gmail.com

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ABSTRACT

Two simple, rapid, precise and reproducible UV spectroscopic methods has been developed for simultaneous estimation of two component drug mixture of doxofylline (DOX) and ambroxol hydrochloride (AMB) in bulk and combined tablet dosage form. First method employs simultaneous equation method using 274nm (λ max of DOX) and 244.5nm (λ max of AMB) as two wavelengths for estimation. The second method involves absorbance correction method the wavelength used were 274nm (λ max of DOX) and 308nm (second λ max of AMB it is zero for DOX). For the two methods distilled water was used as solvent. Linearity was observed in the concentration range of 7 - 35 µg/ml for DOX and 1-5 µg/ml for AMB. The percentage recovery was found in the range of 99.64-100.07 for doxofylline and 98.48-100.55 for ambroxol hydrochloride. The developed method was validated statistically and by recovery studies. The % RSD value was found to be less than 2. Thus the proposed method was simple, precise, economic, rapid and accurate and can be successfully applied for simultaneous determination of doxofylline and ambroxol hydrochloride in bulk and combined tablet dosage form.

Keywords: Doxofylline, Ambroxol hydrochloride, Simultaneous equation, Absorbance correction method, ICH guidelines.

INTRODUCTION

Doxofylline (DOX) is a novel bronchodilator, chemically it is 7-(1, 3-Dioxolan-2-ylmethyl)-3, 7-dihydro-1, 3-dimethyl-1H-Purine-2, 6-Dione[1]. Various analytical methods have been reported for the assay of doxofylline alone. They include UV spectroscopy[2], high performance liquid chromatography[3], high performance thin layer chromatography[4] and LC-MS/MS[5].

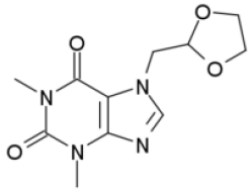

Fig. 1: Chemical structure of doxofylline

Ambroxol hydrochloride is chemically, 1 ({[2 – Amino – 3, 5 dibromo phenyl] –methyl} amino) cyclohexanol monohydrochloride which is a semi synthetic derivative of vasicine from the Indian shrub "*Adhatoda vasica*". It is a mucolytic agent. Ambroxol hydrochloride is an N – desmethyl metabolite of bromohexine[6,7]. Methods such as UV spectroscopy[8-14], high performance liquid chromatography[15-20] ,high performance thin layer chromatography[21,22] and UPLC[23] are reported for estimation of ambroxol hydrochloride alone or in combination with other drugs.

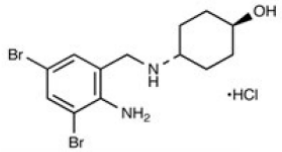

Fig. 2: Chemical Structure Of Ambroxol Hydrochloride

Both the drugs are available in combined tablet dosage form, as an antiasthmatic agent. The extensive literature survey revealed that numbers of methods are reported for the individual drugs but no method is so far reported for the simultaneous estimation of both the drugs in combined pharmaceutical dosage forms. So the present article discusses the attempts made to develop two simple, sensitive and reproducible methods for the simultaneous estimation of DOX and AMB in tablet formulation using simultaneous equation and absorbance correction method[24].

MATERIALS AND METHODS

Instrumentation

The present work was carried out on Shimadzu-1700 double beam UV-Visible spectrophotometer with pair of 10 mm matched quartz cells. Glassware's used were of 'A' grade and were soaked overnight in a mixture of chromic acid and sulphuric acid, rinsed thoroughly with double distilled water and dried in hot air oven.

Reagents and chemicals

Pharmaceutically pure sample of DOX and AMB were generously gifted by Shine Pharmaceuticals Pvt Ltd. Chennai and Apex Pharmaceuticals Pvt Ltd. Allathur. Combination product containing 400mg doxofylline and 30mg ambroxol hydrochloride. All solvents were of AR grade obtained from Qualigens India Pvt. Limited, Mumbai.

Experimental condition

According to the solubility characteristics, the common solvent for the two drugs was found to be distilled water.

Preparation of standard stock solution

Accurately weighed drug samples of both DOX and AMB (50 mg each) were transferred to a suitable standard volumetric flask separately, dissolved and diluted to mark with distilled water. Both the drug solutions were 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 overlain spectra was recorded.

Method A: Simultaneous Equation Method

From the overlain spectra of DOX (10 µg/ml) and AMB (10 µg/ml) in distilled water [Fig 3] wavelengths 274nm (λ max of DOX) and 244.5nm (λ max of AMB) were selected for the formation of Simultaneous equation method. From the above stock solution, aliquots were drawn and suitably diluted so as to get the final concentration range of 7 – 35 µg/ml of DOX and 1-5 µg/ml of AMB.

Absorbances of these solutions were recorded in the respective wavelengths. Both the drugs were linear in the concentration range of 7 – 35 µg/ml of DOX and 1-5 µg/ml of AMB and Calibration curves [n=5] were plotted between concentration and absorbances of drugs with correlation coefficient value not less than 0.999. Optical and regression characteristics are found out. E (1%, 1cm) is determined for DOX at 274 and 244.5nm were 351.69 and 88.964 while respective values for AMB are 319 and 212.5. These values are the mean of six independent determinations.

The simultaneous equations formed were,

At λ_1 $A_1 = a_{x1}bc_x + a_{y1}bc_y$ ------ (1)

$A1 = 351.690C_X + 319.0\ C_Y$------ (2)

At λ_2 $A_2 = a_{x2}bc_x + a_{y2}bc_y$------- (3)

$A2 = 88.964\ C_X + 212.5C_Y$ ------ (4)

Where A_1 and A_2 are the absorbances of sample solution at 274nm and 244.5nm respectively. Cx and C_Y are the concentration of DOX and AMB respectively (µg/ml) in sample solution.

The absorbances [A_1& A_2] of the sample solution were recorded at 274 and 244.5nm 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.

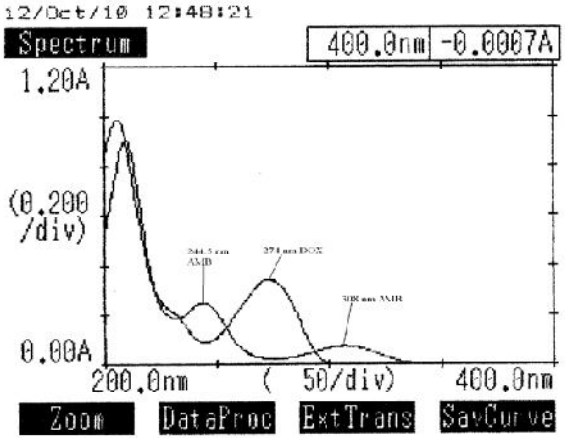

Fig. 3: Overlain zero order spectrum of DOX and AMB

Method B: Absorbance Correction Method

From the overlain spectrum of DOX and AMB in distilled water, it was observed that DOX have zero absorbance at 308 nm, where as AMB has substantial absorbance. Thus AMB was estimated directly at 308 nm without interference of DOX. For estimation of AMB, the absorbance of DOX was measured at 274nm using standard solution of AMB (10 µg/ml). The contribution of AMB was deducted from the total absorbance of sample mixture at 274nm. The calculated absorbance was called as corrected absorbance for DOX. To estimate the amount of DOX, the absorbance of AMB were corrected for interference at 274nm by using absorptivity values. A set of two equations were framed using absorptivity coefficients at selected wavelengths.

$$cx = \frac{A1}{ax1} \qquad cy = \frac{A2 - ax2\ cx}{ay2}$$

Where,

A1 and A2 are absorbance of sample solution at 308nm and 274nm, respectively.

ax1 and ax2 absorptivity coefficients of DOX at 308nm and 274nm, respectively.

ay1 and ay2, absorptivity coefficients of AMB at 308nm and 274nm, respectively.

From the above stock solution, aliquots were drawn and suitably diluted so as to get the final concentration range of 7 – 35 µg/ml of DOX and 1-5 µg/ml of AMB. Absorbances of these solutions were recorded in the selected wavelengths.

Analysis of tablet formulation

Twenty tablets were weighed and average weight was found. The tablets were triturated to a fine powder. An accurately weighed quantity of powder equivalent to 70 mg of DOX was transferred in to 100ml volumetric flask, sufficient 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 14 µg/ml of DOX and 1 µg/ml of AMB with distilled water. The absorbance of sample solution was measured at all selected wavelengths. The content of DOX and AMB in sample solution of tablet was calculated. This procedure was repeated for six times.

Table 1: Results of analysis of tablet formulation

Parameters	DOX		AMB	
	Method A	Method B	Method A	Method B
Labeled claim (mg)	400 mg	400 mg	30 mg	30 mg
% Assay*	99.97	100.32	98.64	99.80
SD	0.32750	2.0275	0.13841	0.1959
%RSD	0.00109	0.5052	0.46779	0.6550

*Mean of six determinations

Validation of methods

The methods were validated with respects to linearity, LOD (Limit of detection), LOQ (Limit of quantitation), precision and accuracy and ruggedness[15].

Linearity

Linearity was checked by diluting standard stock solution at five different concentrations. DOX was linear with the concentration range of 7-35 µg/ml and AMB showed linearity in the range of 1-5 µg/ml and calibration curves [n=5] were plotted between concentration and absorbance of drugs. Optical parameters were calculated.

Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) parameters were calculated, in accordance with ICH guidelines, LOD=3.3σ/S and LOQ=10σ/S respectively, where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

Accuracy

To check the accuracy of the developed method and to study the interference of formulation excipients, analytical recovery experiments were carried out by using standard addition method in three different concentrations. From the total amount of drug found, the percentage recovery was calculated. This procedure was repeated for three times for each concentration. The % RSD was calculated.

Precision

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

Ruggedness

The ruggedness test of analytical assay method is defined as the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different labs, different analysis, different lots of reagents etc. Ruggedness is a measure of reproducibility of test results under normal expected operational conditions from laboratory to laboratory and from analyst to analyst. In present study, determination of DOX and AMB were carried out by using different instruments and different analysts.

Table 2: Spectral and Linearity Characteristics Data

Parameters	DOX		AMB	
	Method A	Method B	Method A	Method B
λmax nm	274nm	274nm	244.5nm	300nm
Linearity range (µg/ml)	7-35	7-35	1-5	1-5
Correlation coefficient (r^2)	0.9999	0.9999	0.9994	0.9993
Molar absorptivity (L mol^{-1} cm^{-1})	9330.737	9330.737	14467.40	4068.786
Sandell's sensitivity (µg/cm^2/0.001A.U)	0.028723	0.028723	0.029669	0.088475
Slope (m)	0.034833	0.034833	0.031782	0.011726
Intercept (c)	0.002097	0.002097	0.001124	0.000166
LOD(µg/ml)	0.216615	0.216615	0.129641	0.053809
LOQ(µg/ml)	0.656410	0.656410	0.392853	0.163058
Standard Error	0.000465	0.000465	0.000233	9.3797E-05

*Mean of six determinations

Table 3: Results of recovery studies

Method	Drug	Amount in µg/ml		% Recovery	S.D*	% RSD*
		Added*	Recovered*			
Simultaneous equation method	DOX	11.2	11.2079	100.07	0.0113	0.1005
		14.0	13.9972	99.90	0.0121	0.0867
		16.8	16.8049	100.02	0.0008	0.0048
	AMB	0.8	0.7874	98.42	0.0012	0.1526
		1.0	0.9990	99.86	0.0037	0.3659
		1.2	1.1935	99.46	0.0022	0.1848
Absorbance correction method	DOX	11.2	11.1975	99.97	0.0043	0.0391
		14.0	13.9496	99.64	0.0044	0.0317
		16.8	16.8012	100.00	0.0087	0.0521
	AMB	0.8	0.8044	100.55	0.0050	0.6172
		1.0	1.0005	100.06	0.0049	0.4904
		1.2	1.1968	99.73	0.0048	0.4101

*Mean of three observations

Table 4: Intermediate Precision And Ruggedness Of The Method

Parameters	%Label Claim Estimated [Mean±%RSD]			
	DOX		AMB	
	Method A	Method B	Method A	Method B
Intraday Precision [n=3]	99.88 ± 0.064	100.21 ± 0.166	90.76 ± 0.087	99.42 ± 0.504
Interday Precision [n=3]	99.95 ± 0.076	100.18 ± 0.239	98.84 ± 0.285	99.54 ±0.639
Different instruments [n=6]				
Instrument I	99.66 ± 0.099	99.89 ± 0.09	90.68 ± 0.26	99.03 ± 0.659
Instrument II	99.97 ± 0.082	100.32 ± 0.505	98.64 ± 0.467	99.60 ± 0.655
Different analyst [n=6]				
Analyst I	99.88 ± 0.096	99.95 ± 0.136	90.91 ± 0.234	99.30 ± 0.721
Analyst II	99.90 ± 0.130	99.99 ± 0.152	98.67 ± 0.260	99.31 ± 0.720

RESULTS AND DISCUSSION

The proposed methods for simultaneous estimation of DOX and AMB in combined dosage form were found to be accurate, simple and rapid. Hence it can be used for routine analysis of two drugs in combined dosage forms.

There was no interference from tablet excipients was observed in these methods. The values of % RSD and correlation of coefficient for simultaneous determination (Tablet) were found to be (% RSD 0.0048 0.617) and correlation coefficient was 0.9999 for DOX and 0.9994 for AMB. The result of recovery studies for tablet was found to be in the range of 98.48 -100.07% for method A, 99.64-100.55 for method B. Values are reported in Table 3. It indicates that there is no interference due to excipients present in the formulation. It can be easily and conveniently adopted for routine quality control analysis. Both methods are accurate, simple, rapid, precise, reliable, sensitive, reproducible and economic and are validated as per ICH guidelines.

CONCLUSION

The results indicate that the proposed UV spectrophotometeric methods are simple, rapid, precise and accurate. The developed UV spectrophotometeric methods were found suitable for determination of DOX and AMB as bulk drug and in marketed tablet dosage formulation without any interference from the excipients. Statistical analysis proves that, these methods are repeatable and selective for the analysis of DOX and AMB.

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The authors are thankful to the management and the principal of Adhiparasakthi College of Pharmacy for providing the necessary facilities to carry out the research work and also thankful to Shine Pharmaceuticals Pvt Ltd. Chennai and Apex Pharmaceuticals Pvt Ltd. Allathur for providing gift samples of the drugs.

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Simultaneous Estimation of Metolazone and Spironolactone in Combined Tablet Dosage Form BY UV Spectroscopy.

UMADEVI.B* and VETRICHELVAN.T

**Department Of Pharmaceutical Analysis,
Adhiparasakthi College of Pharmacy, Melmaruvathur, 603 319. (TN) India.**

*Corres.author: umadevipharm@gmail.com
Mobile No: +919843587007*

Abstract: Three simple, rapid, precise and reproducible UV spectroscopic methods for simultaneous estimation of two component drug mixture of Metolazone(METO) and Spironolactone(SPIR) in combined dosage form have been developed. First method employs simultaneous equation method using 236.5 nm (λ max of METO) and 242.5 nm (λ max of SPIR) as two wavelengths for estimation. The second method involves absorbance correction method, the wavelength used were 242.5nm (λ max of SPIR) and 345nm (second λ max of METO it is zero for SPIR). The third method involves first derivative spectroscopy using 266 nm and 289 nm as zero crossing points for METO and SPIR respectively. For the entire three methods methanol followed by 0.02M phosphate buffer pH 3.5 adjusted with orthophosphoric acid was used. Linearity was observed in the concentration range of 0.5 - 2.5µg/ml for METO and 5-25µg/ml for SPIR. Accuracy and precision of the method was determined by performing intra day and inter day studies results found were satisfactory and statistical validation reveals that they can be applied to marketed samples. Method showed good reproducibility and recovery, this is evident from % RSD which is less than 2%.The methods were successfully applied for determining the amount in marketed formulation.

Key words: Metolazone, Spironolactone, simultaneous equation, first order, zero crossing, absorbance correction.

Introduction

Metolazone (METO) is an Antihypertensive and Diuretic agent chemically it is 7-chloro-1, 2, 3, 4-tetrahydro-2-methyl-4-oxo-3-o-tolyl-6-quinazoline sulfonamide[1-2]. Few HPLC and other methods have been reported for its estimation[3-4]. Spironolactone (SPIR) is a Diuretic drug (Aldosterone antagonist). Chemically it is 7α-acetyl thio-3-oxo-17α pregn-4-ene-21, 17 β-carbolactone[5]. Few estimations in body fluids, bulk in combination with other drugs and in single dosage forms have been reported[6 7]. Both these drugs are available in combined tablet dosage form, as a diuretic agent. The extensive literature survey revealed that numbers of methods are reported for the individual drugs but no method is so far reported for the simultaneous estimation of both the drugs in combined pharmaceutical dosage forms. So the present article discusses the attempts made to develop three simple, sensitive and reproducible methods for the simultaneous estimation of METO and SPIR in table formulation, using simultaneous equation, absorbance correction and first derivative[8].

Developed spectroscopic methods are for simultaneous estimation of METO and SPIR from combined tablet dosage form. Proposed methods are found to be simple, rapid, precise, accurate and reproducible. These methods can be applied successfully for quality

control testing of drugs from combined tablet dosage form, without prior separation.

Experimental

Materials and Methods

Standard bulk drug samples of METO and SPIR were provided by Centaur Pharmaceuticals Mumbai. Tablets of combined dosage form were procured from the local market (METOLACTONE-5). All other reagents used were of analytical grade. Shimadzu UV/ visible spectrophotometer, model 1700 and 1cm matched quartz cells was used. Spectra were recorded using program having following specifications, spectral bandwidth 1 nm, wavelength accuracy ± 0.5 nm, and wavelength readability in 0.1 nm increments.

Method I: Simultaneous Equation Method:

Accurately weighed drug samples of both METO and SPIR (50 mg each) were transferred to a suitable standard volumetric flask dissolved and diluted to mark with methanol. Both the drug solutions were diluted so as to get 10 mcg/ml by using 0.02M phosphate buffer pH 3.5 adjusted with orthophosphoric acid. These solutions were scanned in the UV region of 200-400 nm. From the overlain spectra of METO (10 µg/ml) and SPIR (10 µg/ml) in 0.02M phosphate buffer pH 3.5 adjusted with orthophosphoric acid (**Fig 1**), wavelengths 236.5 nm (λ max of METO) and 242.5 nm (λ max of SPIR) were selected for the formation of Simultaneous equation method. From the above stock solution, aliquots were drawn and suitably diluted so as to get the final concentration range of 0.5 – 2.5 µg/ml of METO and 5-25 µg/ml of SPIR. Absorbances of these solutions were recorded in the said wavelengths. Linearity was found to obey in the said range for both the drugs in both the wavelengths with correlation coefficient value not less than 0.999. Optical and regression characteristics are found out. E (1%, 1cm) determined for METO at 236.5 and 242.5 nm were 128.22 and 102.7 while respective values for SPIR are 402.06and 426.33.These values are the mean of six independent determinations.

The simultaneous equations formed were,

At λ_1 $A_1 = a\,x_1 bc_x + ay_1 bc_y$ ------ (1)

$A1 - 128.22\,C_X + 102.06\,C_Y$ ------ (2)

At λ_2 $A_2 = a\,x_2 bc_x + ay_2 bc_y$ ------- (3)

$A2 - 102.7\,C_X + 426.33 C_Y$ ------ (4)

Where A_1 and A_2 are the absorbances of sample solution at 236.5 and 242.5 nm respectively. Cx and C_Y are the concentration of METO and SPIR respectively (µg/ml) in sample solution.

The absorbances of the sample solution (A_1& A_2) were recorded at 236.5 and 242.5nm 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.

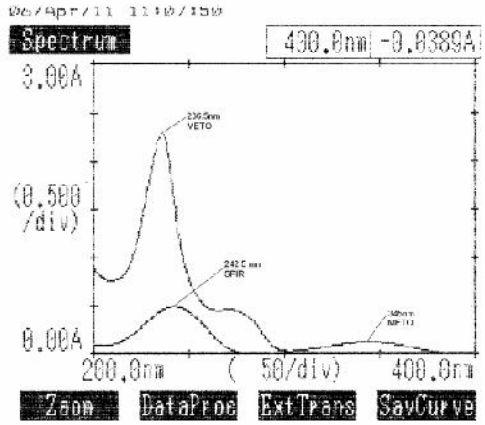

Fig 1. Overlain spectra of zero order spectrum of METO and SPIR

Method II : Absorbance Correction Method:

The standard stock solutions METO and SPIR were further diluted with 0.02M phosphate buffer pH 3.5 adjusted with orthophosphoric acid to get the concentration of 10 µg/ ml of each and the solutions were scanned between the range 200 - 400 nm in 1cm cell against 0.02M phosphate buffer pH 3.5 adjusted with orthophosphoric acid as blank and the overlain spectra was recorded. From the overlain spectrum of METO and SPIR in methanol, it was observed that SPIR have zero absorbance at 345 nm, where as METO has substantial absorbance. Thus METO was estimated directly at 345 nm without interference of SPIR. For estimation of SPIR, the absorbance of METO was measured at 242.5 nm using standard solution of METO (10 µg/ ml). The contribution of METO was deducted from the total absorbance of sample mixture at 242.5 nm. The calculated absorbance was called as corrected absorbance for SPIR. To estimate the amount of SPIR, the absorbance of METO were corrected for interference at 242.5 nm by using absorptivity values. A set of two equations were framed using absorptivity coefficients at selected wavelengths.

$$cx = \frac{A1}{ax1}$$

$$cy = \frac{A2 - ax2\ cx}{ay2}$$

Where,

A1 and A2 are absorbance of sample solution at 345 nm and 242.5 nm, respectively.

ax1 and ax2 absorptivity coefficients of METO at 345 nm and 242.5 nm, respectively.

ay1and ay2, absorptivity coefficients of SPIR at 345 nm and 242.5 nm, respectively.

From the above stock solution, aliquots were drawn and suitably diluted so as to get the final concentration range of 0.5 – 2.5 µg/ml of METO and 5-25 µg/ml of SPIR. Absorbances of these solutions were recorded in the said wavelengths.

Method III: Derivative Spectroscopy determination:

UV spectrum of both the drugs (METO and SPIR) were derivatised to first order with $\Delta\lambda = 1$ for the entire spectrum. Zero crossing points for METO and SPIR was found to be 266 and 289 nm respectively (**Fig 2**). From the above stock solution, aliquots were drawn and suitably diluted so as to get the final concentration range of 1 – 7 µg/ml of METO and 10-70 µg/ml of SPIR 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 was 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).

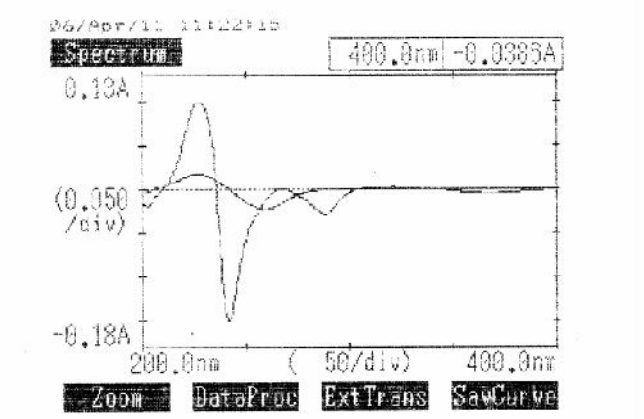

Fig 2. Overlain spectra of first order spectrum of METO and SPIR

Analysis of Commercial Formulations:

Twenty tablets were weighed and average weight per tablet was determined. Tablets were grounded to fine powder and accurately weighed the tablet powder equivalent to 75 mg of METO transferred to the flask, sufficient methanol was added sonicated for 5 min and diluted to the mark with 0.02M phosphate buffer pH 3.5 adjusted with orthophosphoric acid. It was filtered through Whatman Filter paper no: 41, filtrate was suitably diluted to get final concentration with 0.02M phosphate buffer pH 3.5 adjusted with orthophosphoric acid, so as to get the mid concentration of the linearity. Absorbances were measured at the said wavelengths,236.5 and 242.5 nm in Simultaneous Equation method, 266 & 289 nm in First order spectrum for derivative method and at 242.5 and 345 for absorbance correction method and amount present was calculated using simultaneous equation, first order derivative methods and absorbance correction method. Findings are tabulated in table 3.

Validation of methods

The methods were validated with respects to linearity, LOD (Limitof detection), LOQ (Limit of quantitation), precision and accuracy[9].

Recovery studies

To study accuracy, reproducibility and precision of the proposed methods, recovery studies were carried out by standard addition method. Results of recovery studies were found to be satisfactory and presented in Table 4. Precision of the method was determined by performing Intra Day (n = 3) and Inter Day (n = 3) refer the results in table 2.

Table: 1 Spectral and linearity characteristics data of both drugs in Zero Order Spectra.

Parameters	Simultaneous equation method				Asorbance correction method
	236.5nm		242.5 nm		345nm
	METO	SPIR	METO	SPIR	METO
Linearity range(µg/ml)	0.5 -2.5	5 -25	0.5 -2.5	5-25	0.5 -2.5
Correlation coefficient (r^2)	0.9998	0.9999	0.9995	0.9999	0.9999
Molar absorbitivity L mol^{-1} cm^{-1}	46448.87	16782.83	36809.60	17749.80	8093.600
Sandell's Sensitivity (µg/cm^2/0.001A.U)	0.007885	0.024829	0.0100613	0.023654	0.045643
Slope (m)	0.1268571	0.040395	0.1004990	0.0425457	0.022137
Intercept (c)	0.0010785	-0.001104	0.0011761	0.0006063	-0.0001380
LOD(µg/ml)	0.0489834	0.356395	0.076456	0.6907166	0.0530679
LOQ (µg/ml)	0.1484345	1.079985	0.231685	2.093654	0.1608119
Standard error	0.0003785	0.000692	0.0004739	0.0016894	3.82133E-05

Table: 1a Spectral and linearity characteristics data of both drugs in First Order Spectra

Parameters	First derivative method	
	289nm	266nm
	METO	SPIR
Linearity range(µg/ml)	1 -7	10 -70
Correlation coefficient (r^2)	0.9998	0.9999
Molar absorbitivity L mol^{-1} cm^{-1}	682.4295	350.1093
Sandell's Sensitivity (µg/cm^2/0.001A.U)	0.5370689	1.2193285
Slope (m)	0.0018646	0.0008202
Intercept (c)	6.94444E-06	0.0002013
LOD(µg/ml)	0.0276462	0.4184765
LOQ (µg/ml)	0.0837765	1.2681107
Standard error	7.0918E-05	0.00015477

Table: 2 Results of Intraday and Inter day studies

DRUG	Simultaneous equation method	Absorbance correction method
	Average % Found □ S.D,%RSD	**Average % Found □ S.D,%RSD**
	INTRA DAY STUDIES (n = 3)	
METO	99.866□ 0.050, 1.002	100.533 □ 0.045, 0.913
	100.466 □ 0.048, 0.964	100.333 □ 0.045, 0.914
	100.566□ 0.058, 1.169	100.033 □ 0.039, 0.793
SPIR	100.516 □ 0.102, 0.203	99.83 □ 0.099, 0.199
	100.396□ 0.104, 0.209	100.106 □ 0.096, 0.192
	100.426□ 0.117, 0.234	100.283 □ 0.105, 0.210
	INTER DAY STUDIES (n = 3)	
METO	100.366 □ 0.057, 1.151	100.166 □ 0.047, 0.956
	100.166 □ 0.051, 1.021	100.3 □ 0.048, 0.966
	100.333 □ 0.054, 1.089	100.1 □ 0.032, 0.641
SPIR	100.446 □ 0.115, 0.228	100.06 □ 0.094, 0.188
	100.54 □ 0.124, 0.248	99.766 □ 0.125, 0.251
	100.39 □ 0.115, 0.229	100.12 □ 0.085, 0.174

DRUG	First order derivative method Average % Found □ S.D,%RSD
	INTRA DAY STUDIES (n = 3)
METO	100.433□ 0.070, 1.399
	100.233 □ 0.063, 1.264
	100.433□ 0.070, 1.399
SPIR	99.786□ 0.155, 0.310
	100.013□ 0.155, 0.309
	99.836 □ 0.153, 0.307
	INTER DAY STUDIES (n = 3)
METO	99.666□ 0.064,1.298
	100.3 □ 0.067, 1.360
SPIR	100.2□ 0.063, 1.274
	100.093 □ 0.163, 0.326
	99.9461 □ 0.419, 0.840
	99.973 □ 0.155, 0.310

Table: 3 Results of Analysis of Formulation

Methods	Drug	Label Claim	% Label claim found *	S.D *	% RSD *
Simultaneous equation method	METO	5 mg	100.366	± 0.057	1.151
	SPIR	50 mg	100.446	± 0.115	0.2289
Absorbance correction	METO	5 mg	100.166	± 0.047	0.9568
	SPIR	50 mg	100.06	± 0.094	0.1887
First Order Derivative	METO	5 mg	100.2	± 0.063	1.2749
	SPIR	50 mg	99.973	± 0.155	0.3100

*Average of six determinations

Table: 4 Results of Recovery Studies

Method	Drug	Amount in µg/ml		% RECOVERY	S.D*	% RSD*
		Added*	Recovered*			
Simultaneous Equation method	METO	0.8	0.7973	99.67	0.0015	0.1948
		1	0.9995	99.953	0.0026	0.2632
		1.2	1.2031	100.256	0.0063	0.5285
	SPIR	8	7.9531	99.413	0.0101	0.1281
		10	10.032	100.32	0.0174	0.1743
		12	12.1634	101.36	0.0157	0.1294
Absorbance correction	METO	0.8	0.7996	99.95	0.0068	0.8596
		1	1.0029	100.29	0.0068	0.6853
		1.2	1.2001	100.006	0.0025	0.2164
	SPIR	8	8.05	100.626	0.0231	0.28711
		10	10.0535	100.533	0.0207	0.2067
		12	12.0625	100.52	0.01	0.0832
First Order Derivative	METO	2.4	2.4221	100.923	0.0309	1.2776
		3	2.9942	99.806	0.0536	1.7901
		3.6	3.602	100.056	0.0309	0.8591
	SPIR	24	24.0371	100.15	0.0703	0.2927
		30	30.0921	100.306	0.1219	0.4052
		36	36.1065	100.293	0.0703	0.1949

*Average of three determinations

Results and Discussion

Three simple simultaneous estimation methods were successfully developed for the estimation of METO and SPIR in raw material and combined dosage form.

Linearity

Calibration curves were prepared for both the drugs at the selected analytical wavelengths are summarized in Table1 and 1a.This shows that METO obeys Beer's law in the concentration range of 0.5-2.5 µg/ml and SPIR obeys Beer's law in the concentration range of 5-25 µg/ml for simultaneous equation method and absorbance correction method. Whereas METO obeys Beer's law in the concentration range of 1-7 µg/ml and SPIR obeys Beer's law in the concentration range of 10-70 µg/ml for First order Derivative spectroscopy.

LOD and LOQ

LOD and LOQ were calculated, in accordance with ICH guidelines, as $3.3\sigma/S$ and $10\sigma/S$, respectively, where σ is the standard deviation of the response(y-intercept) and S is the slope of the calibration plot.

Accuracy

The accuracy of the method was determined by investigating the recovery of METO and SPIR, three levels ranging from 80, 100 &120% of the nominal concentration by standard addition technique.

The results as shown in Table 4 indicate excellent recoveries.

Precision &repeatability

The precision and repeatability of the method was studied by repeating the proposed method three times in a day and the average percentage and RSD values were tabulated and when the experiment was repeated on three different days the average percentage RSD values for determination was tabulated in Table 2. The results confirm the intra day and inter day precision of the method.

All the three methods are suitable for the reliable analysis of commercial formulations containing combinations of METO and SPIR. The methods are simple, precise, rapid and accurate. High percentage recovery shows that the method is free from the interference of excipients used in the formulation.

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