METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF CELECOXIB AND DIACEREIN IN PURE AND COMBINATION BY UV SPECTROPHOTOMETRY AND RP-HPLC

Dissertation Submitted to

The Tamil Nadu Dr. M.G.R. Medical University

Chennai - 600 032.

In partial fulfillment for the award of Degree of

MASTER OF PHARMACY

(Pharmaceutical Analysis)

Submitted by

PEDDIRAJA NAGASAI RAMESH

Register No. 26106128

Under the Guidance of

Dr. (Mrs). D. NAGAVALLI, M. Pharm., Ph.D.,

Professor

Department of Pharmaceutical Analysis

ADHIPARASAKTHI COLLEGE OF PHARMACY

(Accredited by "NAAC", with a CGPA of 2.74 on a four point scale at "B"-Grade)

Melmaruvathur – 603 319.

MAY 2012

CERTIFICATE

This is to certify that the research work entitled **"METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF CELECOXIB AND DIACEREIN IN PURE AND COMBINATION BY UV SPECTROPHOTOMETRY AND RP-HPLC"** submitted to The Tamil Nadu Dr. M.G.R. Medical University in partial fulfillment for the award of the Degree of the MASTER OF PHARMACY (Pharmaceutical Analysis) was carried out by **PEDDIRAJA NAGASAI RAMESH (REG.NO. 26106128)** in the Department of Pharmaceutical Analysis under my direct guidance and supervision during the academic year 2011-2012.

Place: Melmaruvathur

Date:

Dr. (Mrs.) D. NAGAVALLI. M. Pharm., Ph.D.,
Professor,
Department of Pharmaceutical Analysis,
Adhiparasakthi College of Pharmacy,
Melmaruvathur – 603 319.

CERTIFICATE

This is to certify that the dissertation entitled **"METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF CELECOXIB AND DIACEREIN IN PURE AND COMBINATION BY UV SPECTROPHOTOMETRY AND RP-HPLC"** is the bonafide research work carried out by **PEDDIRAJA NAGASAI RAMESH (REG.NO.26106128)** in the Department of Pharmaceutical Analysis, Adhiparasakthi College of Pharmacy, Melmaruvathur which is affiliated to The Tamil Nadu Dr. M.G.R. Medical University under the guidance of **Dr. (Mrs.) D. NAGAVALLI, M. Pharm., Ph.D.,** Department of Pharmaceutical Analysis, Adhiparasakthi College of Pharmacy, during the academic year 2011-2012.

Place: Melmaruvathur

Date:

Prof. (Dr.) T.VETRICHELVAN, M. PHARM., Ph. D.,
Principal & Head,
Department of Pharmaceutical Analysis,
Adhiparasakthi College of Pharmacy,
Melmaruvathur-603 319.

Dedicated to

My

Parents and friends

ACKNOWLEDGEMENT

First and foremost, I wish to express my deep sense of gratitude to **HIS HOLINESS ARULTHIRU AMMA,** President, ACMEC Trust, Melmaruvathur, for his ever growing blessings in each step of the study. I am grateful to **THIRUMATHI LAKSHMI BANGARU ADIGALAR,** Vice President, ACMEC Trust, Melmaruvathur, for given me an opportunity and encouragement all the way in completing the study.

With great respect and honor, I extend my thanks to **Mr. G. B. ANBALAGAN,** Managing Trustee, APCP, Melmaruvathur. His excellence in providing skillful and compassionate spirit of unstinted support to our department for carrying out my research work entitled **"METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF CELECOXIB AND DIACEREIN IN PURE AND COMBINATION BY UV SPECTROPHOTOMETRY AND RP-HPLC".**

I got inward bound and brainwave to endure experimental investigations in model analytical methods, to this extent, I concede my in most special gratitude and thanks to **Dr. (Mrs.) D. NAGAVALLI, M. Pharm., Ph.D., Professor.,** Department of Pharmaceutical Analysis, Adhiparasakthi College of Pharmacy, Melmaruvathur for the active guidance, valuable suggestions and a source of inspiration where the real treasure of my work.

I owe my sincere thanks with bounteous pleasure **Prof. Dr. T.VETRICHELVAN, M.Pharm., Ph.D.,** Principal, Adhiparasakthi College of Pharmacy, Melmaruvathur without his encouragement and supervision, it would have been absolutely impossible to bring out the work in this manner.

I conceitedly take the dispensation to present my special wisdom of thanks to **Mrs. ABHIRAMI, M. Pharm.,** Assistant Professor and **Mr. K. ANANDAKUMAR, M. Pharm.,** Assistant Professor, Department of Pharmaceutical Analysis for their persuasive support and timely lend a hand to complete this work.

My sincere thanks to our lab technicians **Mrs. S. KARPAGAVALLI, D. Pharm., Mr. M. GOMATHI SHANKAR, D. Pharm., Mrs. N. THATCHAYANI, D. Pharm.,** and **Mr. I. KUMAR,** office assistant for their kind help throughout this work.

I extend my great thankful to **Mr. K. Maruthappapandan,** Managing Director, Ideal Analytical and Research Institution, Puducherry - 605 110, for taking IR Spectroscopy and RP-HPLC studies.

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

I am greatly obliged to my father **Mr. P. Sathyanarasimha Rao**, my mother **Mrs. P. Saradha** and my lovable sisters **Ms. P. Lakshmi Ramya** and **P. Sai Sravani** for their inspiration, guidance, moral support, constant prayers for my successful endeavors.

My genuine thanks to **Dr. Ravindra Mittal**, Medical Advisor & Head Regulatory Affairs, Cadila Health Care Limited, Ahmedabad for gift samples.

I express my whole hearted thanks to my friends **Mr. D. Mani Sai Kumar, Mr. Sreerama Rajasekhar, Mr.M. Madhan mohan, Mr. Nethaji, Ms. Vijaya Shanthi** and **Mrs. Bhavyasri** for their help and suggestion throughout the project.

Words are not just enough to express our gratitude to the **LORD ALMIGHTY** who directed me throughout the work. I humbly present this work to the eternal **ALMIGHTY.** Indeed our project is a small work done with the help of primitive persons at heart. So it is our bounded duty to promulgate them individually.

Finally yet importantly, I gratefully forward my affectionate thanks to my family members, friends, which has sustained me a lot in the successful completion of my project work**.**

PEDDIRAJA NAGASAI RAMESH

CONTENTS

LIST OF FIGURES

FIGURE No.	SUBJECT
34.	CHROMATOGRAM FOR ANALYSIS OF FORMULATION (OSTIGARD®) IN MID LEVEL CONCENTRATION-100% (1)
35.	CHROMATOGRAM FOR ANALYSIS OF FORMULATION (OSTIGARD®) IN MID LEVEL CONCENTRATION-100% (2)
36.	CHROMATOGRAM FOR ANALYSIS OF FORMULATION (OSTIGARD®) IN MID LEVEL CONCENTRATION-100% (3)
37.	CHROMATOGRAM FOR ANALYSIS OF FORMULATION (OSTIGARD®) IN HIGH LEVEL CONCENTRATION-110% (1)
38.	CHROMATOGRAM FOR ANALYSIS OF FORMULATION (OSTIGARD®) IN HIGH LEVEL CONCENTRATION-110% (2)
39.	CHROMATOGRAM FOR ANALYSIS OF FORMULATION (OSTIGARD®) IN HIGH LEVEL CONCENTRATION-110% (3)
40.	CHROMATOGRAM FOR 110% RECOVERY OF FORMULATION (OSTIGARD®)
41.	CHROMATOGRAM FOR 120% RECOVERY OF FORMULATION (OSTIGARD®)
42.	CHROMATOGRAM FOR 130% RECOVERY OF FORMULATION (OSTIGARD®)

LIST OF TABLES

LIST OF ABBREVIATIONS USED

%	-	Percentage
% RSD	-	Percentage Relative Standard Deviation
µ	-	Micron
µl	-	Microlitre
$^{\circ}$C	-	Degree Celsius
AUC	-	Area under Curve
CEL	-	Celicoxib
DIA	-	Diacerein
DMSO	-	Dimethyl sulphoxide
Gms	-	Grams
ICH	-	International Conference on Harmonisation
IR	-	Infra Red
LOD	-	Limit of Detection
LOQ	-	Limit of Quantitation
Mcg/ mL	-	Microgram Per Millilitre
mg/tab	-	Milligram Per tablet
min	-	Minute
mL	-	Millilitre
mM	-	Milli Mole
nm	-	Nanometer
pH	-	Negative Logarithm of Hydrogen Ion
RP-HPLC	-	Reverse Phase -High Performance Liquid Chromatography
Rt or t_R	-	Retention Time
S.D	-	Standard Deviation
S.E	-	Standard Error
UV-VIS	-	Ultraviolet - Visible
v/v	-	Volume/Volume

λ	-	Lambda
µg/ mL	-	Microgram Per Millilitre

INTRODUCTION

1. INTRODUCTION

Pharmaceutical analysis is a branch of practical chemistry that involves a series of process for identification, determination, quantification and purification of a substance, separation of the components of a solution or mixture, or determination of structure of chemical compounds. The substance may be a single compound or a mixture of compounds and it may be in any of the dosage form. The substance used as pharmaceuticals are animals, plants, micro organisms, minerals and various synthetic products.

The sample to be analysed is called as analyse and on the basis of size of sample, they can be classified as macro (0.1 g or more), semi micro (0.01 g to 0.1 g), micro (0.001 g to 0.01 g), sub micro (0.0001 g to 0.001 g), ultra micro (below 10-4 g), trace analysis (100 to 10000 ppm). Among all, the semi micro analysis is widely used.

1.1. Analytical chemistry

Types

There are main two types of chemical analysis.

1. Qualitative (identification)

2. Quantitative (estimation)

1. Qualitative analysis is performed to establish composition of natural/synthetic substances. These tests are performed to indicate whether the substance or compound is present in the sample or not. Various qualitative tests are detection of evolved gas, formation of precipitates, limit tests, colour change reactions, melting point and boiling point test etc.

2. Quantitative analytical techniques are mainly used to quantify any compound or substance in the sample. These techniques are based in

(a) The quantitative performance of suitable chemical reaction and either measuring the amount of reagent added to complete the reaction or measuring the amount of reaction product obtained. (b) The characteristic movement of a substance through a defined medium under controlled conditions.

(c) Electrical measurement.

(d) Measurement of some spectrophotometric properties of the compound.

1.2. U.V SPECTROSCOPY

A UV-vis spectrometer is simply a device that detects how much light is absorbed (blocked). First, a reading on a detector measures how much light reaches the detector without a sample present (before the sample in placed between the light source and detector). Then, when the sample in placed in the light path, if molecules (or atoms) absorb light, the detector "sees" a reduction in light reaching it. We call this reduction absorbance.

The instrument used in ultraviolet-visible spectroscopy is called a UV/Vis **spectrophotometer**. It measures the intensity of light passing through a sample (I), and compares it to the intensity of light before it passes through the sample (I_o). The ratio I / I_o is called the *transmittance*, and is usually expressed as a percentage (%T). The absorbance, A, is based on the transmittance:

$$A = - log (\%T / 100\%)$$

Many molecules absorb ultraviolet or visible light. The absorbance of a solution increases as attenuation of the beam increases. Absorbance is directly proportional to the path length, *b*, and the concentration, *c*, of the absorbing species. *Beer's Law* states that

$$\boldsymbol{A = \varepsilon bc},$$

where **ε** is a constant of proportionality, called the *absorbtivity*.

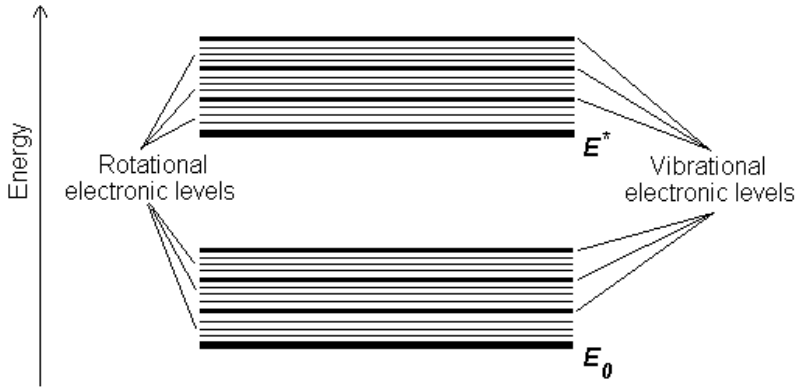
Different molecules absorb radiation of different wavelengths. An absorption spectrum will show a number of absorption bands corresponding to structural groups within the molecule. For example, the absorption that is observed in the UV region for the carbonyl group in acetone is of the same wavelength as the absorption from the carbonyl group in diethyl ketone.

Electronic transitions

The absorption of UV or visible radiation corresponds to the excitation of outer electrons. There are three types of electronic transition which can be considered;

1. Transitions involving σ, π and *n* electrons
2. Transitions involving charge-transfer electrons
3. Transitions involving *d* and *f* electrons

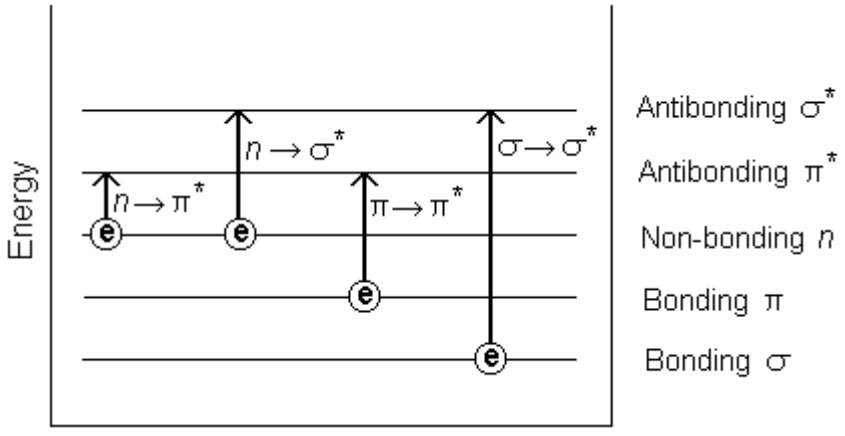
When an atom or molecule absorbs energy, electrons are promoted from their ground state to an excited state. In a molecule, the atoms can rotate and vibrate with respect to each other. These vibrations and rotations also have discrete energy levels, which can be considered as being packed on top of each electronic level.



Absorbing species containing σ, π and *n* electrons

Absorption of ultraviolet and visible radiation in organic molecules is restricted to certain functional groups (chromophores) that contain valence electrons of low excitation energy. The spectrum of a molecule containing these chromophores is complex. This is because the superposition of rotational and vibrational transitions on the electronic transitions gives a combination of overlapping lines. This appears as a continuous absorption band.

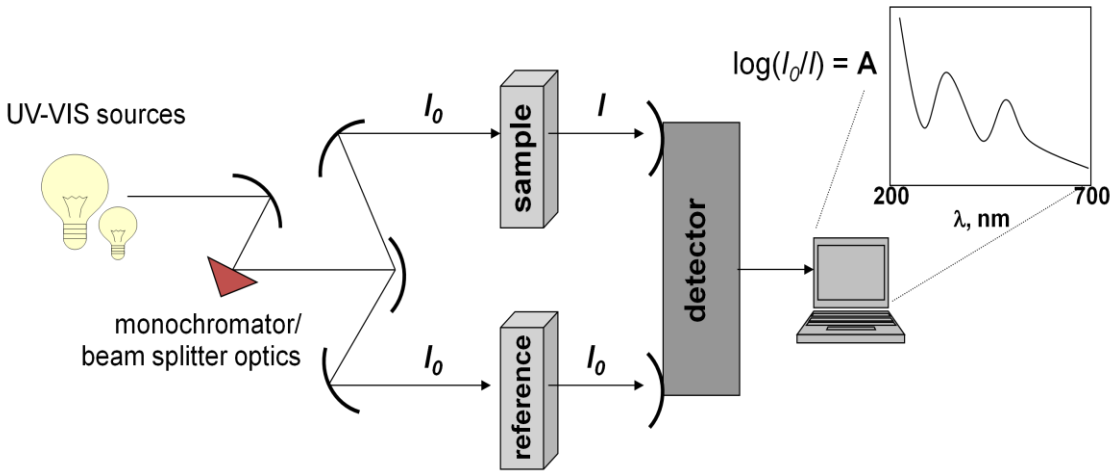
Possible electronic transitions of σ, π and *n* electrons are;



Common solvents and cutoffs:

Acetonitrile	-	190
Chloroform	-	240
Cyclohexane	-	195
1, 4-Dioxane	-	215
95% Ethanol	-	205
N-Hexane	-	201
Methanol	-	205
Isooctane	-	195
Water	-	190

Modern UV – Vis spectrophotometer



The assay of an absorbing substance can be carried out by using

a) Standard absorbtivity value.

b) Use of calibration graph.

c) Single point standardization.

a) Standard absorptivity value:

This procedure is adopted by official compendia for the stable substance that have reasonably broad absorption bands and which are practically unaffected by variation of instrumental parameters. The use of standard A (1%, 1cm) value avoids the need to

prepare a standard solution of the reference substance in order to determine its absorptivity.

b) Use of calibration graph:

In this procedure, the absorbance of a number (typically 4-6) of standard solution of the reference substance at concentrations encompassing sample concentration is measured and a calibration graph is constructed. The concentration of the analyte in the sample solution is read from the graph as a concentration corresponding to absorbance of the solution.

c) Single point standardization

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

$$C_{test} = \frac{A_{test} \text{ x } C_{standard}}{A_{standard}}$$

Where C_{test} and $C_{standard}$ are the concentrations in the sample and standard solutions and A_{test} and $A_{standard}$ are the absorbance of sample and standard solutions respectively.

Important characteristics of spectrophotometric methods include,

- High sensitivities
- Moderate to high selectivity
- Good accuracy
- Wide applicability to both organic and inorganic systems.

- Easy and convenience of data acquisition.

The use of UV and visible spectrophotometer for quantitative analysis employs the method of comparing the absorbance of standards and samples at a selected wavelength. The analysis of mixtures of two or more components is facilitated by activity of absorbance. Other applications include measurement of absorption of complexes to establish their composition. All chromogenic compounds are not suitable for quantitative measurements, i.e. the choice of the system and procedure depends largely on the chemistry of the species to be determined.

Points to be considered in the selection of procedure include:

- Stability of absorbance with respect to time, variation of pH, ionic strength and temperature.
- Degree of selectivity of complexing agent includes the effect of other species likely to be present.
- Conformity to the Beer-Lambert's Law and plot calibration data for the range of concentration measured.

In multi-component formulations, the presence of two or more drugs in a formulation give rise to interference components which mutually interfere with each other in their estimation. For the simultaneous estimation of drugs in such formulations many techniques have been applied.

They are

1) Simultaneous equation method
2) Area under curve method
3) Absorbance ratio method

4) Derivative spectroscopy method.

5) Chemical Derivatization Methods.

6) Multi-component mode of analysis.

SIMULTANEOUS EQUATION METHOD

If a sample contains two absorbing drugs (X and Y) each of which absorbs at the λ_{max} of the other. It may be possible to determine the quantity of both drugs by the technique of simultaneous equation (or) Vierodt's method.

Criteria for obtaining maximum precision, based upon absorbance ratios have been suggested that place limits on the relative concentrations of the component of the mixture.

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

Where, ax_1, ax_2 = Absorptivities of X at λ_1 and λ_2

ay_1, ay_2 = Absorptivities of X at λ_1 and λ_2

A_1, A_2 = Absorbance of the diluted sample at λ_1 and λ_2.

The ratio should lie outside the range of 0.1 – 2.0 for the precise determination of (Y and X) two drugs respectively.

These criteria are satisfactory only when the λ_{max} of the two components is reasonably dissimilar. The additional criteria includes that two components do not interact chemically, there by negating the initial assumption that the total absorbance is the sum of the individual absorbance

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

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

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

DERIVATIVE SPECTROPHOTOMETRIC METHOD

This method involves the conversion of normal spectrum to its first, second or higher derivative spectrum. The transformations that occur in the derivative spectra are understood by reference to a Gaussian band which represents an ideal absorption band.

In this technique spectra are obtained by plotting the first or higher derivation of absorbance or transmittance with respect to wavelength versus wavelength. Often these plots reverse spectral details, with respect to wavelength versus wavelength. Often these plots reverse spectral details, which is lost in an ordinary spectrum. In addition concentration measurements of an analyte in the presence of their interference can sometimes be made easily or more accurately.

A first-order derivative spectrum is a plot of the gradient of the absorption curve (rate of change of the absorbance with wavelength, $dA/d\lambda$) against wavelength.

A second-order derivative spectrum is a plot of the curvature of the absorption spectrum against wavelength ($d^2A/d\lambda^2$). The second derivative at any wavelength λ is related to concentration by the following equations:

$$\frac{\mathrm{d}^2 A}{\mathrm{d}\lambda^2} = \frac{\mathrm{d}^2 A_{1\ \mathrm{cm}}^{1\ \mathrm{per\ cent}}}{\mathrm{d}\lambda^2} \times \frac{c'b}{10} = \frac{\mathrm{d}^2 A\varepsilon}{\mathrm{d}\lambda^2} \times \frac{cb}{10}$$

For quantitative measurements, the amplitude is the distance between the adjacent maxima and minima is usually measured.

Enhanced resolution, band width discrimination are the advantages of derivative spectrophotometry that permit the selective discrimination of certain absorbing substances in samples in which non specific interferences may prohibit the application of simple spectrophotometric methods.

1.3 CHROMATOGRAPHY

Modern pharmaceutical formulations are complex mixtures including' in addition to one or more therapeutically active ingredients, a number of inert materials such as diluents, disintegrants, colours and flavours. In order to ensure quality and stability of the final product, the pharmaceutical analyst must be able to separate the mixtures into individual components prior to quantitative analysis.

Amongst the most powerful techniques available to the analyst for the separation of these mixtures are group of highly efficient methods collectively called chromatography.

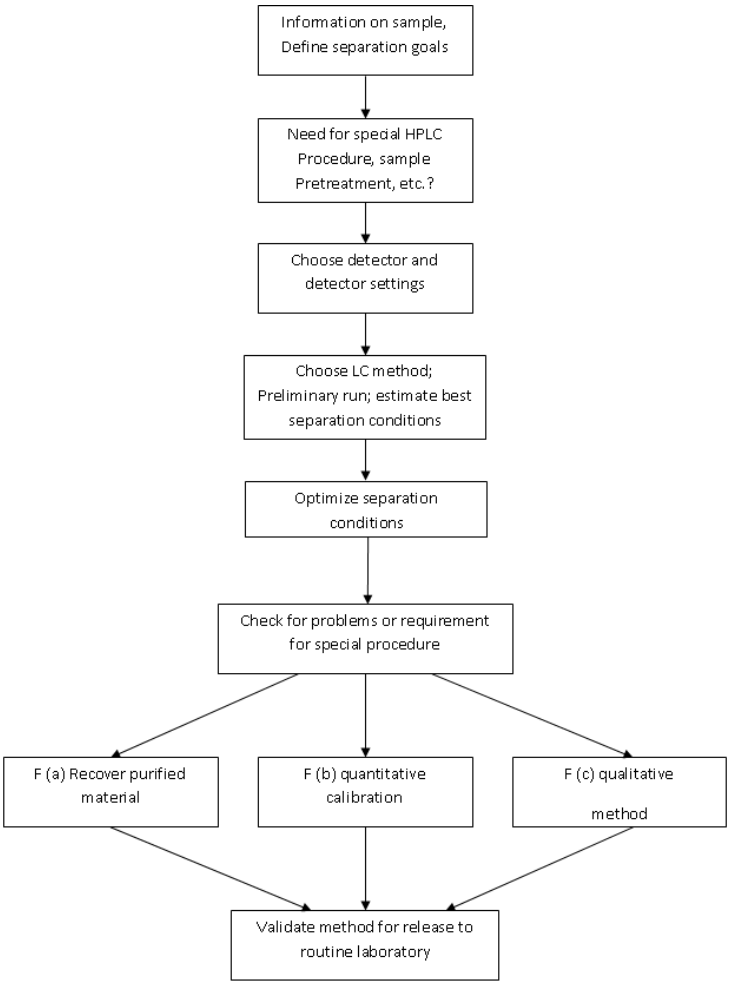
It is a group of technique for the separation of compounds of mixture that depends on the affinities of the solutes between two immiscible phases. One of the phases is

affixed bed of large surface area, while the other is a fluid which moves through the surface of the fixed phase. The fixed phase is called stationary phase, and the other is termed as the mobile phase.

Depending on the type of chromatography employed, the mobile phase may be a pure liquid or a mixture of solutions (Eg. Buffer) or it may be gas (pure or homogenous mixture).

Chromatographic methods can be classified according to the nature of the stationary and mobile phases.

DEVELOPMENT OF THE REVERSE PHASE HPLC METHOD



VALIDATION (Sethi P.D., 2001)

Validation of an analytical method is the process by which it is estimated, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications.

REASONS FOR VALIDATION

1. Enables scientists to communicate scientifically and effectively on technical matters.
2. Setting standards of evaluation procedures for checking complaints and taking remedial measures.
3. Retrospective validation is useful for trend comparison of results compliance to cGMP/GLP.
4. Closer interaction with pharmacopoeia harmonization particularly in respect of impurities determination and their limits.
5. For taking appropriate action in case of non – compliance.
6. To provide high degree of confidence that the same level of quality is consistently built into each unit of finished product from batch to batch.
7. Economic: The consistency and reliability of validated analytical procedure is to produce a quality product with all the quality attributes, thus providing indirect cost saving from reduced testing or re testing and elimination of product rejection.

As quality control process is not static, some form of validation / Verification should continue till the validated process is in use.

Summary of validation procedure

Type of validation	Test for
Specificity	Interference
Accuracy	Recovery ; linearity
Sensitivity	Limit of detection ; Limit of quantification
Precision	Repeatability ; Reproducibility ; Ruggedness
Personnel	Qualifications ; Experience ; responsibility ; proficiency
Equipment	Specifications, vendor, calibration, maintenance
Service	Sanitation, water, Waste disposal

1.4. PARAMETERS USED FOR ASSAY VALIDATION (ICH Guidelines)

Typical analytical characteristics used in method validation are

Specificity

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

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 an accepted reference value and the value found.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same

homogenous sample under the prescribed conditions. Precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

Repeatability

Express the precision under same operating conditions over a short interval of time. Repeatability is also termed as intra-assay precision.

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 in the sample.

Range

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

Detection limit

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Based on the standard deviation of the response and the slope, the detection limit (DL) may be expressed as

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

Where,

σ = standard deviation of the response

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

Quantification limit

The quantification limit of an analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision, accuracy and reliability by the proposed method.

Based on the standard deviation of the response and the slope, quantitation limit may be expressed as

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

Where,

σ = standard deviation of the response

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

1.5. System suitability parameters

System suitability is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such.

The system suitability testing parameters established for the liquid chromatographic procedure are:

Retention time (R_t)

This is the time of emergence of the maximum of a component after injection.

Symmetry factor (or) tailing factor (T)

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

The assessment of peak shape is in terms of symmetry factor.

Number of theoretical plates (N)

$$N = 5.54\left[\frac{t}{W_{\frac{h}{2}}}\right]^2$$

The assessment of performance of efficient of a column is in terms of the number of theoretical plates.

Resolution

$$\frac{2(t_2 - t_1)}{W_2 + W_1}$$

1.6. Pharmaceutical Statistics

Statistics consist of a set of methods and rules for organizing and interpreting observations.

The precision or reproducibility of the analytical method was determined by repeating the analysis six times and the following statistical parameters were calculated.

The Formulas are

$$\text{Standard Deviation} = \sqrt{\frac{\Sigma(x - \bar{x})^2}{n - 1}}$$

$$\text{R.S.D (\%)} = \frac{\text{S.D}}{\bar{x}} \text{ X } 100$$

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

Where	Σ	=	Sum of observations
	$\bar{x}$	=	Mean or arithmetic average ($\Sigma x / n$)
	x	=	Individual observed value
	$x - \bar{x}$	=	Deviation of a value from the mean
	n	=	Number of observations

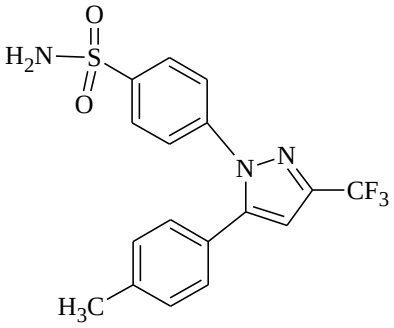
LITERATURE REVIEW

2. LITERATURE REVIEW

2.1 DRUG PROFILE

2.1.1 CELECOXIB (The Merck Index 2006, www.wikipedia.com)

Molecular structure



Chemical name

4[5(4-methylphenyl)-3-[trifluoromethyl]pyrazol-1yl-benzene sulfonamide

Molecular formula

$C_{17}H_{14}F_3H_3O_2S$

Molecular weight

381.373 gm/ mol

Category

Celecoxib is an NSAID, a selective inhibitor of cyclo-oxygenase-2 (COX-2). Celecoxib is used in the treatment of rheumatoid arthritis and osteoarthritis.

Primary characteristics

It is of synthetic origin and belongs to NSAID.

Identification

i) Melting Point

Standard Value	Observed Value*
158-162° C	161.16° C

*****Average of six determination

Pharmacokinetics

Volume of distribution is found to be 500 l/ kg and plasma protein binding is 97%. Renal Excretion accounts for < 1% and plasma half life is ~97.

Indications

Celecoxib is primarily indicated in conditions like osteoarthritis, rheumatoid arthritis, and can also be given in adjunctive therapy as an alternative drug of choice in dysmenorrhoea, pain, and toothache.

Side effects

The severe or irreversible adverse effects of Celecoxib, which give rise to further complications, include GI bleeding, Rhinitis, Myalgia, Urinary tract infection, Bronchitis, Pharyngitis. The symptomatic adverse reactions produced by Celecoxib are more or less tolerable and if they become severe, they can be treated symptomatically, these include Flatulencce, Dizziness, Headache, Vomiting, Diarrhoea, Constipation, Insomnia, Abdominal pain, Rashes, Pruritus, Dyspepsia, Cough.

Warning and precautions

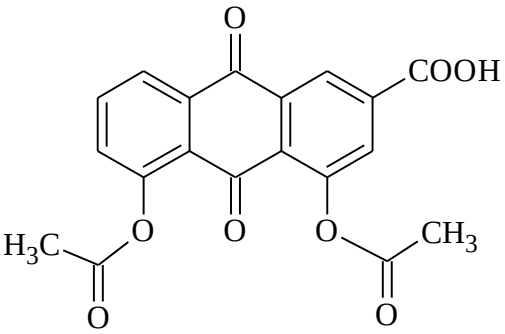
It is classified as pregnancy category C, and should be used with caution during pregnancy only if clearly needed. Avoid use in case of hypersensitivity to Celecoxib or any component, sulphonamides, aspirin or other non steroidal anti-inflammatory drugs.

Storage

Capsules: Store in a well closed container, Between 20°C-25°C. Protect from Moisture and Heat.

2.1.2 DIACEREIN (Indian Pharmacopeia, 2010 and The Merck Index, 2006)

Molecular structure



Chemical name

9,10-dihydro-4,5-dihydroxy-9,10-dioxo-2-anthranoic acid diacetate

Molecular formula

$C_{19}H_{12}O_8$

Molecular weight

368.294

Category

Antirheumatic, it is used in the treatment of osteoarthritis and chronic inflammatory arthritis.

Description

Fine yellow power

Solubility

Freely soluble in dimethyl sulfoxide (DMSO)

Identification

i) Melting Point

Standard Value	Observed Value*
217-218° C	217.83° C

*****Average of six determination

Storage

Store protected from light.

Absorption, Distribution, Metabolism and Elimination

Absorption:

Oral bioavailability of the diacerein is 35% to 56% concurrent intake of food delays the time to peak concentration from 2.4 hours to 5.2 hours, but it is associated with a 25% increase in absorption. Therefore, diacerein is best given with food.

Distribution:

The total protein binding of rhein, a metabolite is about 99% to plasma albumin and γ-immunoglobulins. It achieves synoviral fluid concentration of 0.3 to 3.0 mg/ L

Metabolisum:

Diacerein is metabolised completely (100%) in liver following oral dosing prior to entering systemic circulation. The cell localization of this deacetylation has not been definitively identified, but plasma can be ruled out. Major active metabolites include rhein sulfate with half life being 7 to 8 hours.

Elimination:

Diacerein is excreted in urine in the form of its metabolites (35-60%), with approximately 20% as free rhein and 80% as conjugates of rhein.

Contra indication:

a) Hypersensitivity to diacerein of any components of the product.

b) Combined use of diacerein with laxatives is contraindicated.

Side effects:

Nausea, dry mouth, constipation, diarrhoea, vomiting, decrease appetite, dizziness, headache, increased sweating, hot flushes and anxiety.

Drug interactions:

Diacerein given with food shown increase in absorption by 25% the drug combinely used with laxatives may cause interactions, hence contraindicated.

2.2. LITERATURE REVIEW

2.2.1 REPORTED METHOD FOR CELECOXIB

1. Jayasagar G., *et al*., (2002) reported validated HPLC method for determination of Celecoxib in human serum and it applications in pharmacokinetic study. This chromatography achieved on C_{18}, Wakosil column with a mixture of 10 M Potassium dihydrogen ortho phosphate (pH 3.2) and acetonitrile (50:50 v/v) at 250 nm.
2. Saha RN., *et al*., (2002) reported determination of Celecoxib in pharmaceutical formulations using UV spectroscopy and liquid chromatography. The linear regression equations are obtained by least square regression method were ABS= 4.949 x 10 (-2). Conc. (in ng/ ml) +1.110 x 10(-2) for the UV method and Area under the curve=5.340 x 10(1). Conc. (in ng/ ml) +3.144 x 10(2) for the LC method.
3. Neelamseedher *et al*., (2003) reported spectrophotometric method for estimation of some COX-2 inhibitors in pure form in pharmaceutical formulations. UV spectrophotometric method for estimation of Celecoxib, Rofecoxib, Meloxicam and Nimusulide in pure form of pharmaceutical formulations solvents employed for method 0.1 N sodium hydroxide.
4. Chandran S., *et al*., (2006) reported rapid and sensitive spectrofluorimetric method for estimation of Celecoxib and Flurbiprofen. The excitation and emission wavelengths were found to be 256 nm and 403 nm respectively for Celecoxib in water and 250 nm and 314 nm respectively for flurbiprofen in 1:1 mixture of methanol and 0.1 N H_2SO_4. The linear regression equation obtained by least square regression method for fluorescence intensities (F_5) and concentration in mg/ mL.

5. Murthy TEGK., *et al*., (2006) reported reverse phase HPLC determination of Celecoxib in dosage forms using ODS column in mixture of 0.2 % v/v of glacial acetic acid and acetonitrile (32:68 v/v) at 260 nm.
6. Archana Roy., *et al*., (2008) reported "Development of rapid UV spectrophotometric methods for estimation of Celecoxib and Acyclovir in formulations". The λ determination for Celecoxib was determined in 0.1 N HCl with 1% w/v, SLS at 255 nm. And that of acyclovir in 0.1 N HCl at 257 nm.

2.2.2. REPORTED METHOD FOR DIACEREIN

1. Giannellini V., *et al*., (2005) reported "A validated HPLC stability-indicating method for determination of Diacerein in bulk drug substance". This chromatography method was achieved on a RP_{18} (End capped coloum) utilizing 0.1 M phosphoric acid and methanol (40:60 v/v) at 254 nm.
2. Keddal G. Lalitha., *et al*., (2009) reported a simple HPLC method for quantitation of in tablet dosage form. This chromatographic method was achieved on ZorbaxCN column with a mixture of ammonium acetate buffer (pH adjusted to 3.5): Acetonitrile (53:47) at 254 nm.
3. Janhavi Rao., *et al*., (2009) reported "Stability-Indicating high performance liquid chromatographic method for the determination of Diacerein in capsules"**.** This chromatographic separation was achieved on ODS C_{18} column with mobile phase mixture of Acetonitrile: phosphate buffer (60: 40 % v/v) pH4 adj with phosphoric acid at 254 nm.
4. Sarika NArade., *et al*., (2010) reported "Development and Validation of UV Spectrophotometric method for determination of Diacerein capsules". Diacerein

shows maximum absorbance at 258.5 nm with molar absorbtivity of 4.2258 x 10^4 l/mol/cm.

5. Sekar V., *et al*., (2010) reported "Development and validation of RP-HPLC method for simultaneous estimation of Diacerein and Aceclofenac in dosage form. This chromatographic method was achieved on Phenomenex Luna C_{18} 5µ, 250 mm x 4.6 mm column, using dipotassium hydrogen phosphate buffer of pH 4.5 acetonitrile and methanol in ratio of 40:40:20 at 260 nm.
6. Kannappan N., *et al*., (2010) reported "Analytical method development and Validation of Diacerein tablets by RP-HPLC". This chromatographic method was achieved on Zorbax CN, the mobile phases were comprised of Acetonitrile and Buffer pH-3.5.
7. Siva Kumar R., *et al*., (2010) "Visible spectrophotometric estimation of Diacerein in bulk and formulation form". Method A is based on the reaction of Diacerein with Folin-Ciocalteu reagent, in the presence of 0.5 N sodium hydroxide solution, giving a pink-colored chromogen, which shows maximum absorbance at 512 nm against reagent blank while method B is based on the oxidation of Diacerein with potassium permanganate in an alkaline medium giving a pink-colored chromogen, which shows maximum absorption at 497.5 nm.
8. Sreejith K.R., *et al*., (2010) "Novel Spectrophotometric Methods for Estimation of Diacerein from Formulations". Method I was developed using solvent pyridine with minimum processing steps which showed maximum absorbance at 435 nm. In Method II a spectrophotometric method was developed using 0.1N sodium hydroxide which showed λ_{max} at 503 nm.

PLAN OF

WORK

3. AIM AND PLAN OF WORK

The combination dosage form selected for the present study contains CELECOXIB and DIACEREIN in solid oral dosage forms, recently this combination of the drugs introduced into market.

In the view of the literature cited for the quantification of CELECOXIB and DIACEREIN it was felt that a rapid and accurate method for estimation of CELECOXIB and DIACEREIN individually and in combination with other drugs was available, but no method is available in combined dosage form. The present work is aimed to develop a method for estimation of the drugs in combined capsule dosage form.

For UV method,

1. find the drugs solubility in various solvents
2. to determine maximum absorbance and overlaid
3. determining the standard absorbance for all selected wavelength for each drugs
4. development of simple, precise, accurate and sensitive
   - Simultaneous equations method
   - Area under curve method using (Simultaneous equation)
   - Derivative method, in the specified range
5. Validation of development as per ICH guidelines

For RP-HPLC method,

1. Selection of mobile phase for suitable for two drugs which proper resolution with possible short duration time
2. While selecting mobile phase cost be consider to make this method for the route analysis
3. Development of chromatogram with various concentration for each drug to determine range of concentrations
4. Relating area of chromatogram with respect to concentration for individual drugs
5. Development of chromatogram in formulation Validation of the developed method

Materials & Methods

4. MATERIALS AND METHODS

4.1 MATERIALS USED

4.1.1 Drug samples used

CELECOXIB and DIACERIEN were generoumacy gifted by Zydus Cadila Health Care Ltd, Ahmedabad.

4.1.2 Formulation used

(OSTIGARD®100) containing 100mg Celecoxib and 50mg of Diacerein was gifted by Zydus synovial (A division of Cadila Health care Limited)

4.1.3 Chemicals and Solvents used

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

Methanol (HPLC grade)

Acetonitrile (HPLC grade)

Water (HPLC grade)

Methanol (Analytical grade) and

DMSO (Analytical grade)

4.1.4 Instruments Used

Instruments employed for the study were,

- SHIMADZU AUX - 220 Digital Balance
- SHIMADZU – 1700 Double Beam - UV –Visiblspectrophotometer with pair of 10 mm matched quartz cell
- Thermal scientific spectra HPLC
- ELICO SL 159 Spectrophotometer
- ELICO pH meter (Model LI - 120)
- Melting point apparatus

4.1.5 Instruments Specifications

1) Shimadzu AX – 200 digital balance: (Shimadzu instruction manual)

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

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

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

Specifications	
Light source	20 W halogen lamp, Deuterium lamp. Light source position automatic adjustment mechanism.
Monochromator	Aberration-correcting concave holographic grating
Detector	Silicon Photodiode
Stray Light	0.04% or less (220 nm: NaI 10 g/ L) 0.04% or less (340 nm: $NaNo_2$ 50 g/ L)
Measurement wavelength range	190-1100 nm
Spectral Band Width	1 nm or less (190 to 900 nm)
Wavelength Accuracy	± 0.5 nm automatic wavelength calibration mechanism
Recording range	Absorbance : -3.99~3.99 Abs Transmittance : -399~399%
Photometric Accuracy	± 0.004 Abs (at 1.0 Abs), ±0.002 Abs (at 0.5 Abs)
Operating Temperature/Humidity	Temperature range : 15 to 35° C Humidity range : 35 to 80% (15 to below 30° C) 35 to 70% (30 to 35° C)

C) INSTRUMENT SPECIFICATIONS FOR HPLC

THERMO SCIENTIFIC SPECTRA HPLC SYSTEM	
PUMP	P4000
AUTO SAMPLER	AS 3000
UV –VIS DETECTOR	UV 2000
VACUME DEGASSER	SCM 1000
SYSTEM CONTROLLER	SN 4000
SAMPLE COOLER	UP TO 2- 4° C
SOFT WARE	CHROM QUEST 5.0

4) Sonica ultra sonic cleaner- model 2200 MH

5) ELICO – pH meter model L1610

6) Micropipette

7) Melting point apparatus - Guna enterprises Chennai

4.2 METHODS

Methods employed for UV-spectroscopy

In the present work an attempt was made to develop and validate a simple, precise and accurate method for the estimation of Celecoxib and Diacerein in pure and in combined tablet dosage form by UV spectrophotometry and RP-HPLC method.

4.3 UV SPECTROPHOTOMETRIC METHODS

4.3.1. Simultaneous Equation Method

4.3.2. Area under curve method

4.3.3. Derivative Spectrophotometric Method

4.4. RP-HPLC

4.3. UV SPECTROPHOTOMETRIC METHOD

SELECTION OF SOLVENT

The solubility of drugs was determined in a variety of solvents as per Indian pharmacopoeia standards. Solubility was carried out in polar to non polar solvents. The common solvent was found to be 1mL of DMSO/methanol were chosen as solvent for Spectrophotometry, and it was selected on account of its ready availability, cost factor, and solubility for the analysis of Celecoxib and Diacerein for proposed method.

Preparation of standard stock solution of Celecoxib

10 mg of Celecoxib raw material was weighed accurately and transferred into 10 mL volumetric flask and dissolved in 1 mL D.M.S.O and made up to the volume with methanol. This solution was observed to contain 1000 µg/ mL. From this 2 mL volume of Celicoxib stock is transferred to 25 mL volumetric flask and made up to required volume with methanol to get con concentration of 80 µg/ mL. It is used as working standard.

Preparation of standard stock solution of Diacerein

10 mg of Diacerein raw material was weighed accurately and transferred into 10 mL volumetric flask and dissolved in 1 mL DMSO and made up to the volume with methanol. This solution was observed to contain 1000 µg/ mL. From this 1 mL volume of stock solution is transferred to 25 mL volumetric flask and made up to required volume with methanol to get con concentration of 40 µg/ mL. It is used as working standard.

Selection of wavelength

The selection of wavelengths for the estimation Celecoxib and Diacerein suitable diluted stock solution contain 10 µg/ mL of each and the solutions were scanned between 200 – 400 nm by using methanol as blank. From the overlain spectra, by the observation of spectral characteristics of Celecoxib and Diacerein were selected for simultaneous estimation. The wavelengths selected were 236 nm and 269 nm. For Area under curve 236-236 nm and 267-273 nm were selected as wave lengths form the overlaid spectrum. For Derivative spectrophotometric method, the zero order spectrum was derivatized to first order spectrum in that 294 nm was selected for estimation of Celecoxib, which is zero crossing for Diacerein and 245 nm was selected for estimation of Diacerein which is zero crossing point of Celecoxib

LINEARITY AND CALIBRATION

4.3.1 Simultaneous Equation method

From the working stock solution of Celecoxib (80 µg/ mL), pipette out 0.5 to 2.5 mL into a series of five 10 mL volumetric flask and made up to mark with methanol to get concentration range of 4 to 20 µg/ mL. From the working stock solution of Diacerein

(40 µg/ mL), pipette out 0.5 to 2.5 mL into a series of five 10 mL volumetric flask and made up to mark with methanol to get concentration range of 2 to10 µg/ mL.

The linearity was carried out individually for both the drugs and absorbances of these solutions were measured at 236 nm and 269 nm.

4.3.2 FOR AREA UNDER CURVE METHOD

From the working stock solution of Celicoxib (80 µg/ mL) and Diacerein (40 µg/ mL), pipette out 0.5 to 2.5 mL into a series of five 10 mL volumetric flasks separately and made up to mark with methanol to get concentration range of 4 to 20 µg/ mL for Celecoxib and 2 to 10 µg/ mL for Diacerein .

The linearity was carried out individually for both the drugs and area of these solutions was measured at 236-246 nm and 267-273 nm.

4.3.3 FOR DERIVATIVE SPECTROPHOTOMETRIC METHOD

The $\Delta A/\Delta\lambda$ values were measured in the first order derivative mode. Derivatised values were measured at 294 nm and 245 nm. The calibration curve for all the wavelengths were constructed by plotting $\Delta A/\Delta\lambda$ Vs concentration for first order derivative method.

QUANTIFICATION IN FORMULATION

Twenty Capsules were weighed and average weight of each capsule was found. The contents of the capsules were removed. The content of the drug equivalent to 25 mg of Diacerein was transferred to a 50 mL standard flask and the content of the flask was dissolved in DMSO and methanol by sonication for 15 minutes and made up to the volume and filtered through Whatmann filter paper (No.41). The solution was diluted to get a concentration of 10 µg/ mL in methanol.

Absorbance of the diluted sample solutions were measured at 236 nm and 269 nm for Simultaneous Equation Method, area at 236-246 nm and 267-273 nm for area under curve method and $\Delta A/\Delta\lambda$ 294 and 245 nm for first order derivative Spectrophotometric Method.

Validation of developed method

Linearity

A calibration curve was plotted between concentration and absorbance. Celecoxib was linear with the concentration range of 4 – 20 µg/ mL and Diacerein showed the linearity in the range of 2 – 10 µg/ mL at selected wavelengths for both the methods.

RECOVERY STUDIES

In order to ensure the accuracy of the proposed method, recovery studies were carried out. To 50 % of the pre-analyzed sample solution, a definite concentration of 6.4, 8 and 9.6 µg/ mL standard solution of Celecoxib and 3.2, 4 and 4.8 µg/ mL standard solution of Diacerein were added and then its recovery was studied. The absorbance of resulting solutions was measured at their corresponding wavelengths and the percentage recovery was calculated.

PRECISION

Precision of the method was demonstrated by repeatability studies. Repeatability studies were done by consequently analyzing the sample solution for six times. Intraday and inter day precision were established by repeating the determination on the same day and on different days respectively.

Ruggedness

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

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

The linearity studies were carried out for six times. The limit of detection and limit of quantification were calculated by using the average of slope and standard deviation of intercept.

4.4 REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD

Chromatographic method depends up on the nature of the sample, molecular weight and solubility. The drug selected for the present study was polar compound, hence it can be separated either by normal phase or reverse phase chromatography. Reverse phase chromatographic technique was selected for initial separations with the knowledge of properties of compound, LichroCART 250-4(Lichrospher 100) – RP 18e – (5 μm) column was chosen as stationary phase and various mixtures of methanol and water were considered as mobile phase.

SELECTION OF MOBILE PHASE AND λ_{max}

Different mixtures of mobile phase with various ratios were selected and their chromatograms were recorded. From this methanol:water 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.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

The following parameters were used for RP-HPLC analysis of Celecoxib and Diacerein.

Mode of operation – Isocratic

Stationary phase	–	LichroCART 250-4(Lichrospher 100) – RP 18e – (5 µm) Column
Mobile phase	–	Methanol: Water
Ratio	–	85:15
Detection wavelength	–	252 nm
Flow rate	–	1 mL/ min.
Column Temperature	–	30°C
Sample volume	–	20 µg/ mL
Operating pressure	–	150 kgf

PREPARATION OF THE STANDARD STOCK SOLUTION

Weighed accurately 100 mg of Celecoxib and 50 mg of Diacerein , transferred into a 500 mL standard volumetric flask separately and dissolved with acetonitrile 250 mL and the volume was made up to the mark with methanol. From the above solutions 5 mL were transferred to a 10 mL flask and diluted with methanol to get the concentration of 100 µg/ mL Celecoxib and 50 µg/ mL for Diacerein.

LINEARITY AND CALIBRATION

From the standard solution, pipette 0.7-1.3 mL into a series of seven 10 mL flask and made up to the mark with mobile phase to obtain the concentration range from 70-130 µg/ mL for Celecoxib and 35-65 µg/ mL for Diacerein solution were injected and chromatogram was recorded. The calibration curve was plotted between concentration and peak area.

QUANTIFICATION OF CELECOXIB AND DIACEREIN

Twenty Capsules were weighed and average weight of each capsule was found. The content of the capsules removed. The content of the drug equivalent to 100 mg of Celecoxib which also contains 50 mg of Diacerein was transferred to a 500 mL volumetric flask and the content of flask was dissolved in 250 mL acetonitrile by sonication for 15 minutes and made up to the volume with methanol and filter through Whatmann filter paper (No. 41) and 5 mL was pipetted into a 10 mL volumetric flask and made up to the mark with methanol to produce 10 µg/ mL solutions. The peak area measurements were done by injecting sample (20 µg/ mL) six times and the amount of Celecoxib and Diacerein were calculated from their respective calibration curve.

RECOVERY STUDIES

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

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

The linearity studies were carried out for six times. The limit of detection and limit of quantification were calculated by using the average of slope and standard deviation of response.

SYSTEM SUITABILITY STUDIES

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

RESULTS

AND

DISCUSSION

5. RESULTS AND DISCUSSION

Estimation of multiple drug formulations have advantage that the methods are time consuming and usage of solvent is minimized. Three simple, rapid, precise and accurate spectrophotometric methods and an isocratic RP – HPLC method were developed and validated for the estimation of Celecoxib and Diacerein in pure form and in combined capsule dosage form. The methods employed

5.1 UV-SPECTROPHOTOMETRIC STUDIES

The identification of Celecoxib and Diacerein were confirmed by melting point analysis and IR spectral studies (Fig. 1 and 2)

The solubility of Celecoxib and Diacerein were determined in a variety of solvents using Schefter and Higuchi method. 10 mg samples were taken in test tube and checked their solubility with variety of solvents as per IP and the profiles were shown in Table-1.

The common solvent was found to be 1 mL of DMSO/methanol were chosen as solvent for Spectrophotometry, and it was selected on account of its ready availability, cost factor, and solubility for the analysis of Celecoxib and Diacerein for proposed method. The drugs were stable in DMSO/methanol up to two hours for Celecoxib and Diacerein respectively.

Three simple, sensitive and precise UV methods namely, simultaneous equation method, area under curve and derivative spectrophotometric methods were selected for the determination of Celecoxib and Diacerein in pharmaceutical formulations.

The drugs were dissolved in methanol to produce 10 µg/ mL, they were scanned in the range of 200-400 nm and it shows constant λ_{max} at 236 nm for Celecoxib and 269 nm for Diacerein and overlaid spectra was made. This is shown in Fig-3, 4 and 5 respectively. Stability of absorbance at their λ_{max} was also checked.

The linearity of Celecoxib and Diacerein was constructed in the range of 4-20 and 2-10 µg/ mL and their calibration curves were shown in Fig. 6 to 13 respectively. A simple, accurate, rapid and precise first order derivative method was developed and validated. The common solvent used for estimation of Celecoxib and Diacerein was chosen as 1 mL DMSO and methanol.

The sample solutions of 10 µg/ mL of Celecoxib and Diacerein in 1 mL DMSO and methanol prepared individually and the solutions were scanned in UV region in the wavelength range from 200 to 400 nm by using methanol as blank. The zero order spectrums were derivatized into first order derivative spectrum. The overlaid first order derivative spectrum of Celecoxib and Diacerein was recorded as shown in Figure 14, 15 and 16. From the spectrum, 294 nm and 245 nm were selected for the estimation of Celecoxib and Diacerein respectively without any interference. At 245 nm, Celecoxib has zero absorbance and at 294 nm, Diacerein has zero absorbance value. Hence these two wavelengths were selected for the analysis of Celecoxib and Diacerein, respectively. Different aliquots of Celecoxib and Diacerein were prepared in the concentration range of 4 - 20 µg/ mL and 2 – 10 µg/ mL, respectively. The absorbances of these solutions were measured at 294 nm and 245 nm in the first order derivative spectrum for Celecoxib and Diacerein, respectively. The plotted graphs are shown in Figure 17 and 18 for Celecoxib and Diacerein, respectively.

The optical characteristics such as Beer's law limit (4-20 and 2-10 µg/ mL), Molar extinction co-efficient, Sandell's sensitivity, Correlation co-efficient, Slope and Intercept were calculated and are shown in Table- 2, 7 and 12 respectively.

The limit of detection and the limit of quantification were determined from the linearity studies which was done 6 times and calculated by using slope and standard deviation of response (Intercept).

The formulation **OSTIGARD®** was selected for analysis. The amount present were determined by calculating the average of six replicate analysis and its percentage purity was found to be in the range of 98-101 % by the three methods. It is shown in Table- 3, 8 and 13 respectively.

To evaluate the accuracy of the method, recovery studies were carried out, known amount of pure drug was added to the pre-analyzed solution containing formulation and the mixture was analyzed by the proposed methods, and their recoveries were calculated. The percentage recovery of Celecoxib and Diacerein in the formulation **OSTIGARD®** was found to be in the range of 98-102%. These values are shown in Table- 6, 11 and 16.

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 by two other analysts. The %RSD and standard deviation for inter-day and intra-day analysis was found to be less than 2 indicates the method is precise, which are shown in Table 4, 5, 10, 11, 14 and 16 respectively.

5.2 RP-HPLC METHOD

An effort was made to develop a simple, precise and accurate method for the method development and estimation of Celecoxib and Diacerein in bulk and in combined capsule dosage form by RP-HPLC method.

SELECTION OF MOBILE PHASE

Methanol was preferred because of its lower viscosity and high UV transparency. Methanol was selected due to its inexpensiveness. Methanol: Water in the ratio 85: 15 was selected and this gave sharper peaks with good resolution and it was selected as mobile phase. The base line and individual chromatograms of Celecoxib and Diacerein were recorded shown in Fig 19, 20 and 21 respectively. The detection wavelength was measured by scanning the solution of Celecoxib and Diacerein in mobile phase, in UV spectrophotometry, overlaid spectra and the wavelength of maximum absorption was selected as 252 nm. The limit of detection and the limit of quantification were determined by using slope and standard deviation response. The system suitability parameters such as theoretical plate, Tailing factor, Asymmetric factor and Resolution were calculated and shown in Table-17, the parameters were found to be satisfactory as per guidelines.

With the optimized chromatographic conditions, stock solutions of Celecoxib and Diacerein were prepared in Acetonitrile and methanol (1:1) and prepared the mixture in the concentration range 70-130 µg/ mL of Celecoxib and 35-65 µg/ mL of Diacerein. 20 µL of each solution was injected and records the chromatogram at 252 nm. The chromatogram were recorded and shown in Fig.22 to 28

The calibration curve was plotted using concentration against peak area. The procedure was repeated for three times. The correlation coefficient was found to be above

0.9998 and 9996 for two drugs. The calibration graph of Celecoxib and Diacerein are shown in Fig.29 and 30 characteristics of Celecoxib and Diacerein shown in Table-18.

The capsule dosage form **OSTIGARD®** was selected for the analysis. The ostensible concentration 100 µg/ mL of Celecoxib and 50 µgm/mL of Diacerein in the mobile phase were prepared. 20 µL of each solution was injected and chromatograms were recorded. The percentage purity was found to be 98.98 % Celecoxib and 99.77 % of Diacerein respectively.

The precision of the method was confirmed by repeatability of formulation for nine times and the chromatograms are shown in Fig.31-39. The percentage of RSD was found to be 0.1257 and 0.0878 for Celecoxib and Diacerein respectively. The data is shown in Table-19.The accuracy of the method was performed by recovery studies to the pre - analyzed formulation, a known quantity of Celecoxib and Diacerein raw material solutions were added at different levels, injected the solutions. The chromatograms were recorded as shown in the Fig.40 - 42. The percentage recovery was found to in the range between Celecoxib 99.98% and 99.76% Diacerein. The percentage RSD was found to be 0.305 and 0.387 for Celecoxib and Diacerein respectively. The low percentage of RSD values for recovery indicated that the method was found to be accurate. The values were given in the Table-20. The high percentage recovery revealed that no interference was produced due to the excipients used in formulation. Therefore developed method was found to be accurate. All the above parameters with the ease of operations ensure that the proposed methods could be applied for the routine analysis of Celecoxib and Diacerein pure form and in capsule dosage form.

SUMMARY

AND

CONCLUSION

6. SUMMARY AND CONCLUSION

Celecoxib is chemically 4[5(4-methylphenyl)-3-[trifluoromethyl] pyrazol-1yl-benzene sulfonamide. It is a NSAID, a selective inhibitor of cyclo-oxygenase-2 (COX-2). Celecoxib is used in the treatment of rheumatoid arthritis and osteoarthritis.

Diacerein is chemically 9, 10-dihydro-4, 5-dihydroxy-9, 10-dioxo-2-anthranoic acid diacetate. It is an anti rheumatic and used in the treatment of osteoarthritis and chronic inflammatory arthritis.

Literature survey reveals that there is no analytical method reported for Simultaneous estimation of Celecoxib and Diacerein in pharmaceutical dosage form.

So the present study aims to develop a newer, rapid, accurate and precise analytical method for the Simultaneous estimation of Celecoxib and Diacerein in the formulation of OSTIGARD®.

The proposed analytical methods are simple, economical, rapid, sensitive, reproducible and accurate for the simultaneous estimation of Celecoxib and Diacerein. The methods adopted for studies were,

1. **UV- SPECTROPHOTOMETRIC METHOD**
   - 1.1 Simultaneous equation method
   - 1.2 Area under curve
   - 1.3 Derivative spectrophotometric method
2. **RP-HPLC**

The drug samples containing Celecoxib and Diacerein in combined capsule dosage forms were analyzed by UV-spectrophotometric method using 1 mL DMSO and methanol as a solvent and the contents of drug determined in each formulation was found to be satisfactory.

UV-spectrophotometric method for the estimation of Celecoxib and Diacerein in combined capsule dosage form by

Simultaneous equation method

Area under the curve using simultaneous equation method and

Derivative spectrophotometric method

6.1 UV SPECTROPHOTOMETRIC METHODS

Simultaneous equation method

The percentage label claim present in Capsule formulation was found to be 100.20% and 100.06% for Celecoxib and Diacerein respectively. The percentage recovery was found to be in the range of 99.97-100.90%.

Area under curve

The percentage label claim present in tablet formulation was found to be 100.48% and 100.08% for Celecoxib and Diacerein respectively. The percentage recovery was found to be in the range of 100.71-100.16%.

Derivative spectrophotometric method

The percentage label claim present in tablet formulation was found to be 100.33% and 100.32% for Celecoxib and Diacerein respectively. The percentage recovery was found to be in the range of 100.04-100.16%.

6.2 RP-HPLC method.

RP-HPLC method has been developed for the estimation of both drugs in bulk and in formulation. The proposed method gives reliable assay results with short analysis time, using mobile phase Methanol: Water in the ratio of (85:15). The contents of drug present in the formulation were found to be satisfactory and system suitability parameters are in desired limit.

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

Thus the above studies findings would be helpful to the analytical chemists to apply the analytical methods for the routine analysis of the analyte in pharmaceutical dosage forms after their approval from FDA.

FIGURES

FIGURE – 1

IR SPECTRUM OF CELECOXIB

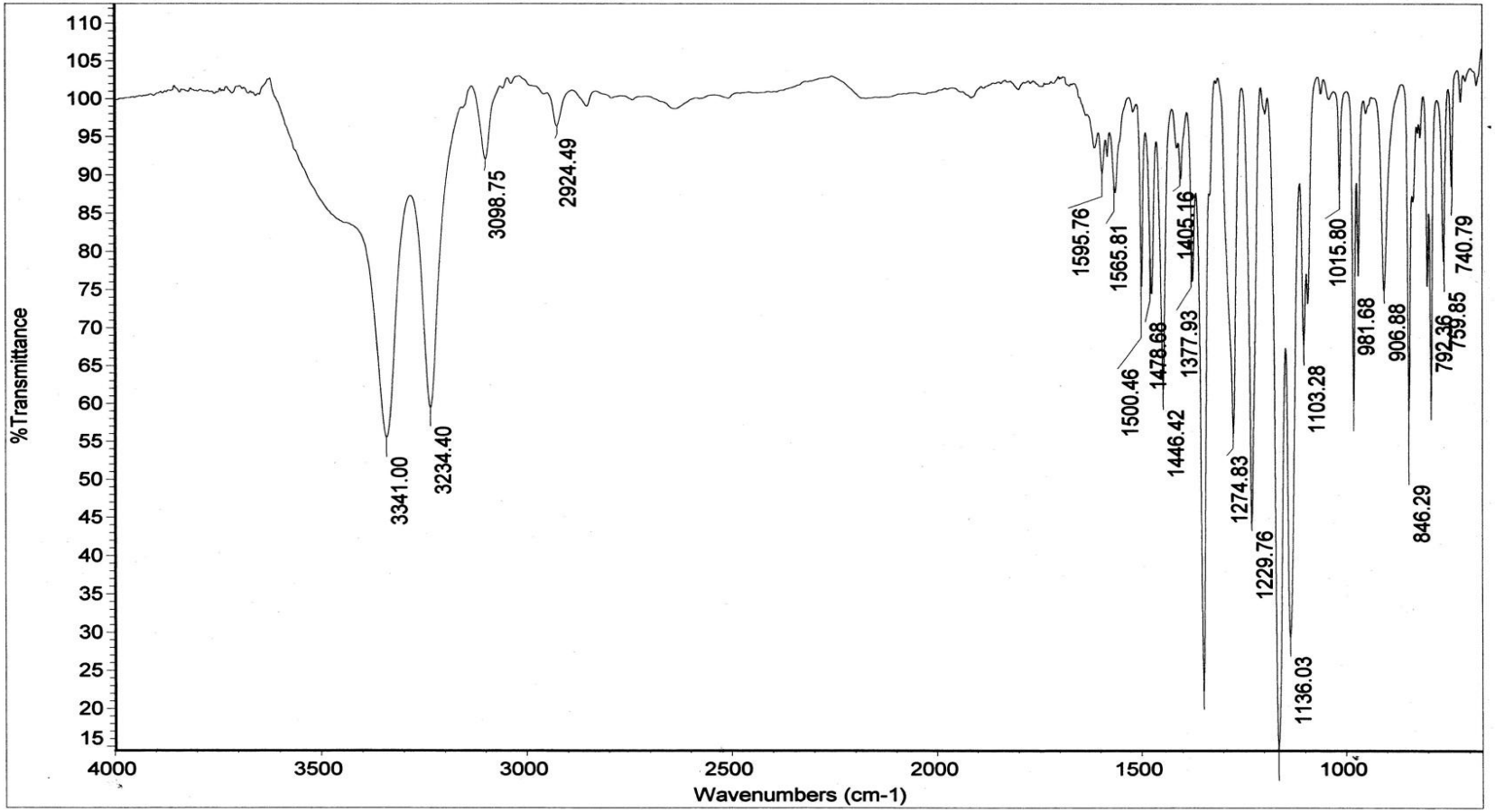


FIGURE – 2

IR SPECTRUM OF DIACEREIN

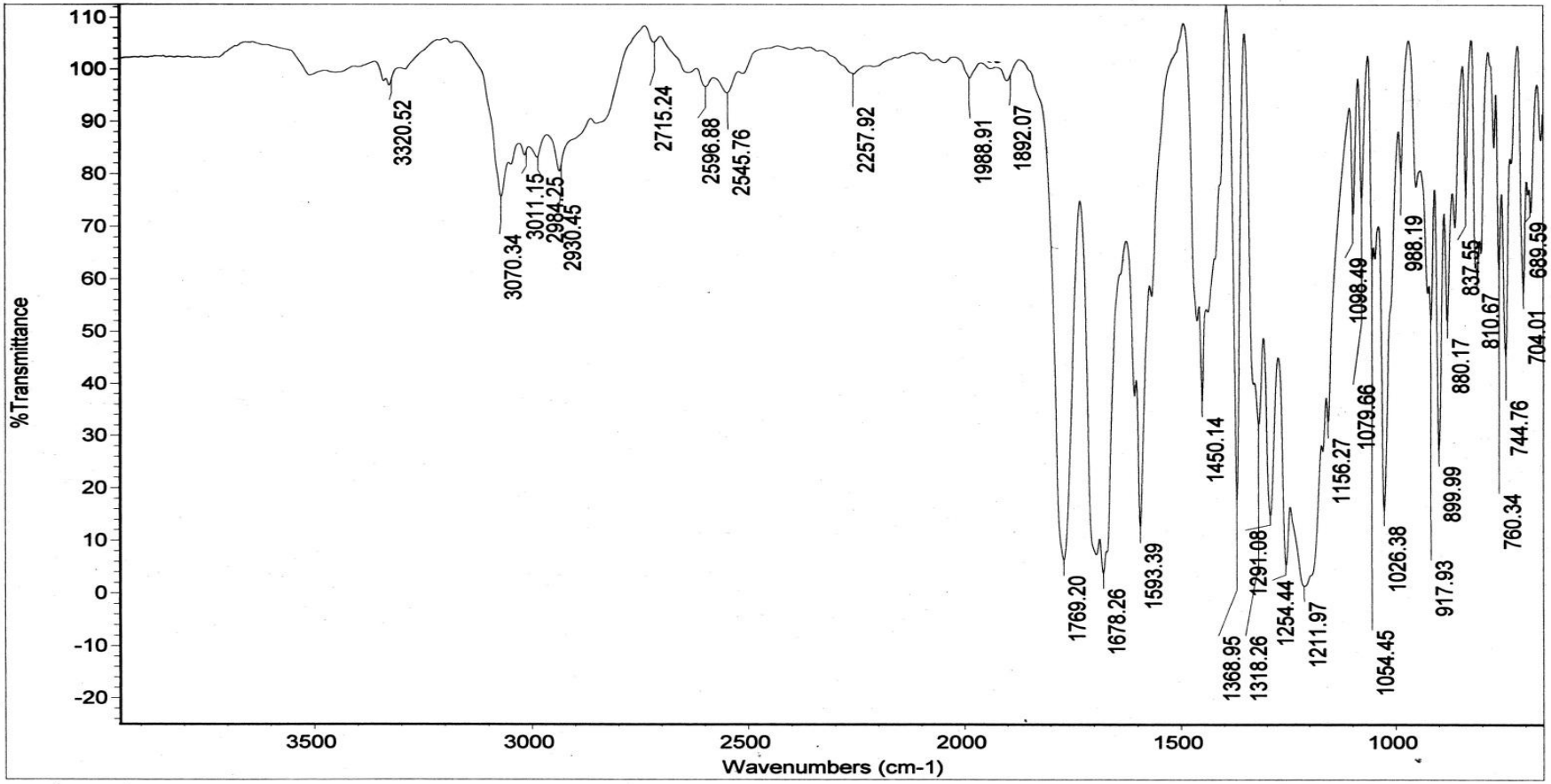
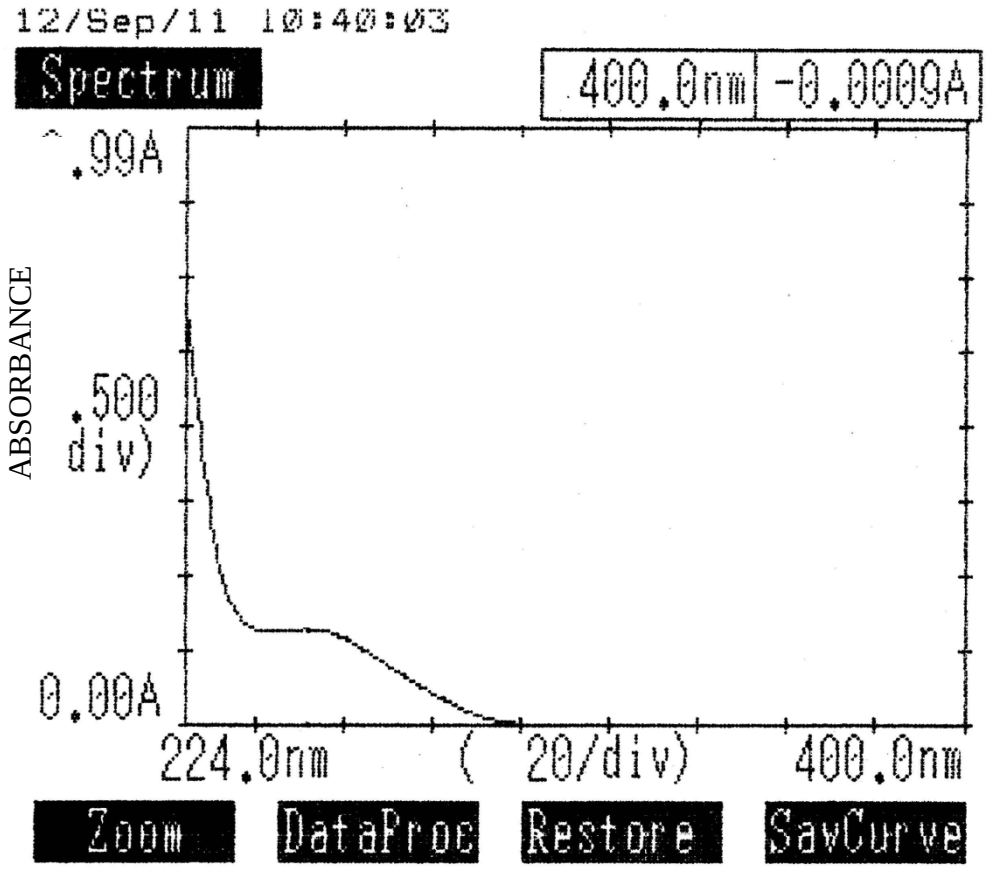


FIGURE – 3

UV SPECTRUM OF CELECOXIB AT

1 mL DIMETHYL SULPHOXIDE AND METHANOL AT 251.5 nm

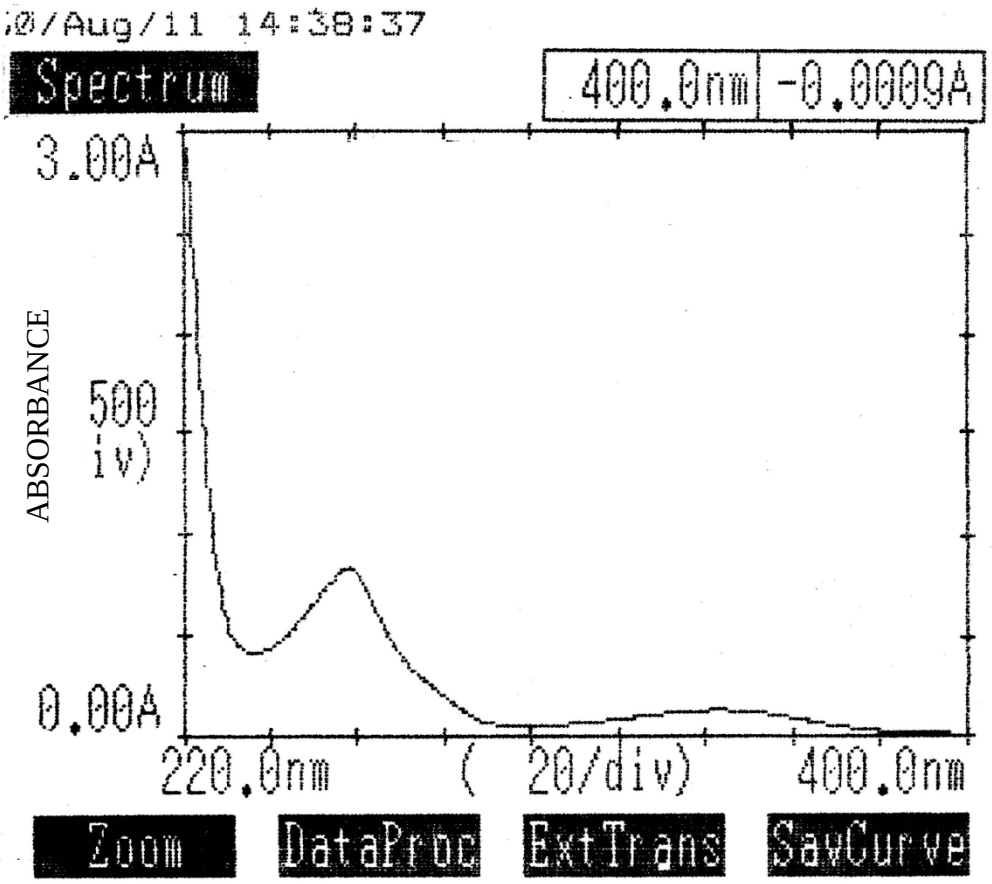


Wavelength (nm)

FIGURE – 4

UV SPECTRUM OF DIACEREIN AT

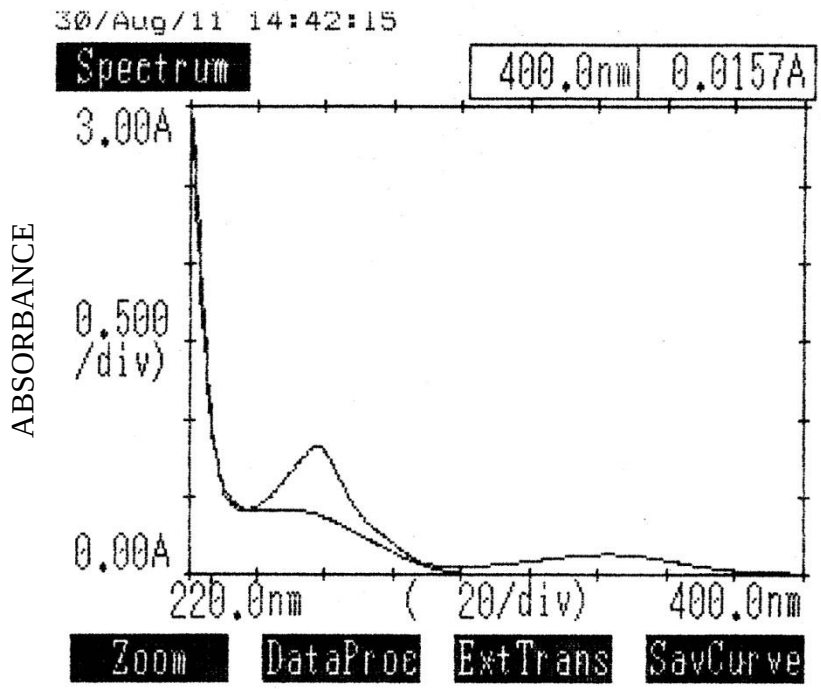
1 mL DIMETHYL SULPHOXIDE AND METHANOL AT 258 nm



Wavelength (nm)

FIGURE – 5

UV OVERLAIND SPECTRUM OF CELECOXIB AND DIACEREIN IN 1 mL DIMETHYL SULPHOXIDE AND METHANOL



Wavelength (nm)

FIGURE – 6

CALIBRATION CURVE OF CELECOXIB IN

1 ml DIMETHYL SULPHOXIDE AND METHANOL AT 236 nm

(SIMULTANEOUS EQUATION METHOD)

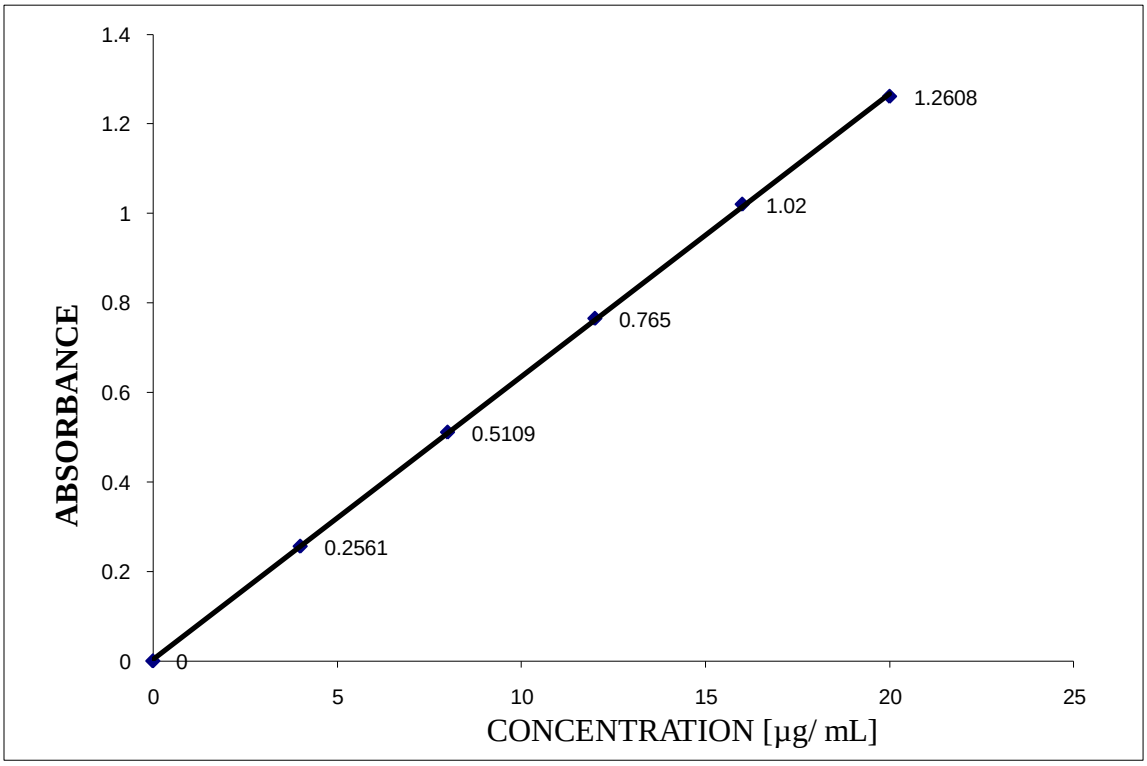


FIGURE – 7

CALIBRATION CURVE OF CELECOXIB IN

1 mL DIMETHYL SULPHOXIDE AND METHANOL AT 269 nm

(SIMULTANEOUS EQUATION METHOD)

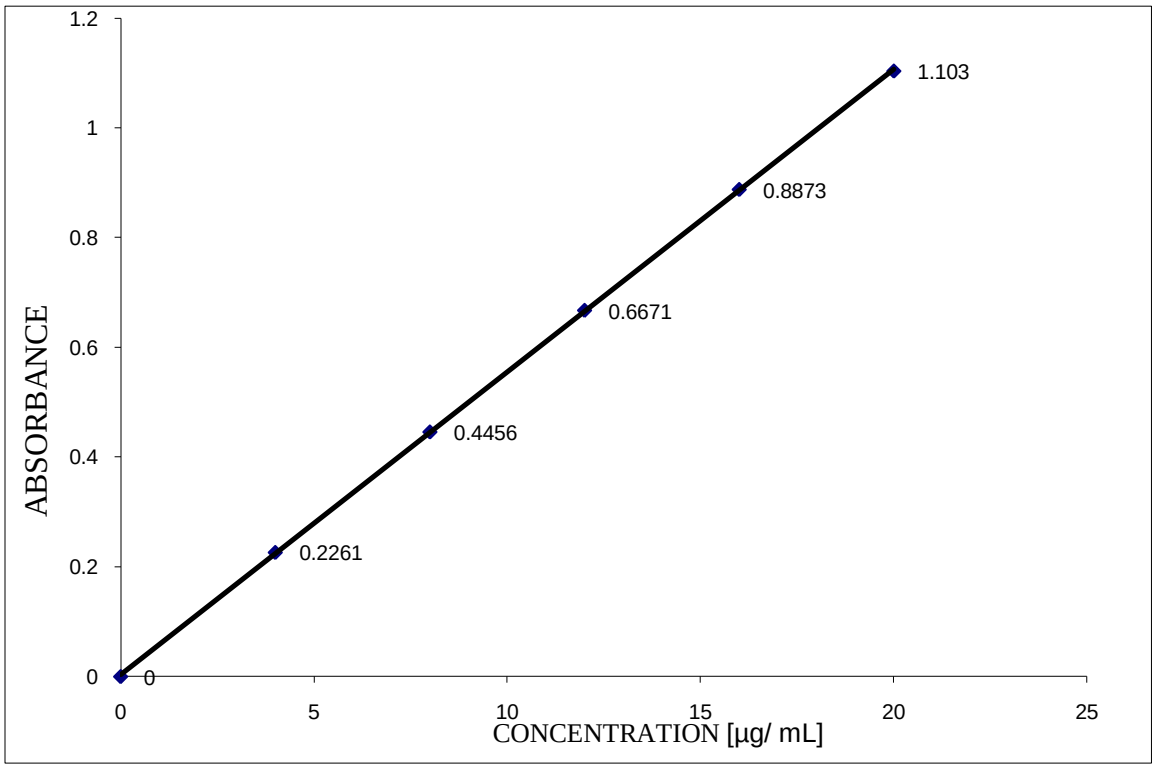


FIGURE – 8

CALIBRATION CURVE OF DIACEREIN IN

1 mL DIMETHYL SULPHOXIDE AND METHANOL AT 236 nm

(SIMULTANEOUS EQUATION METHOD)

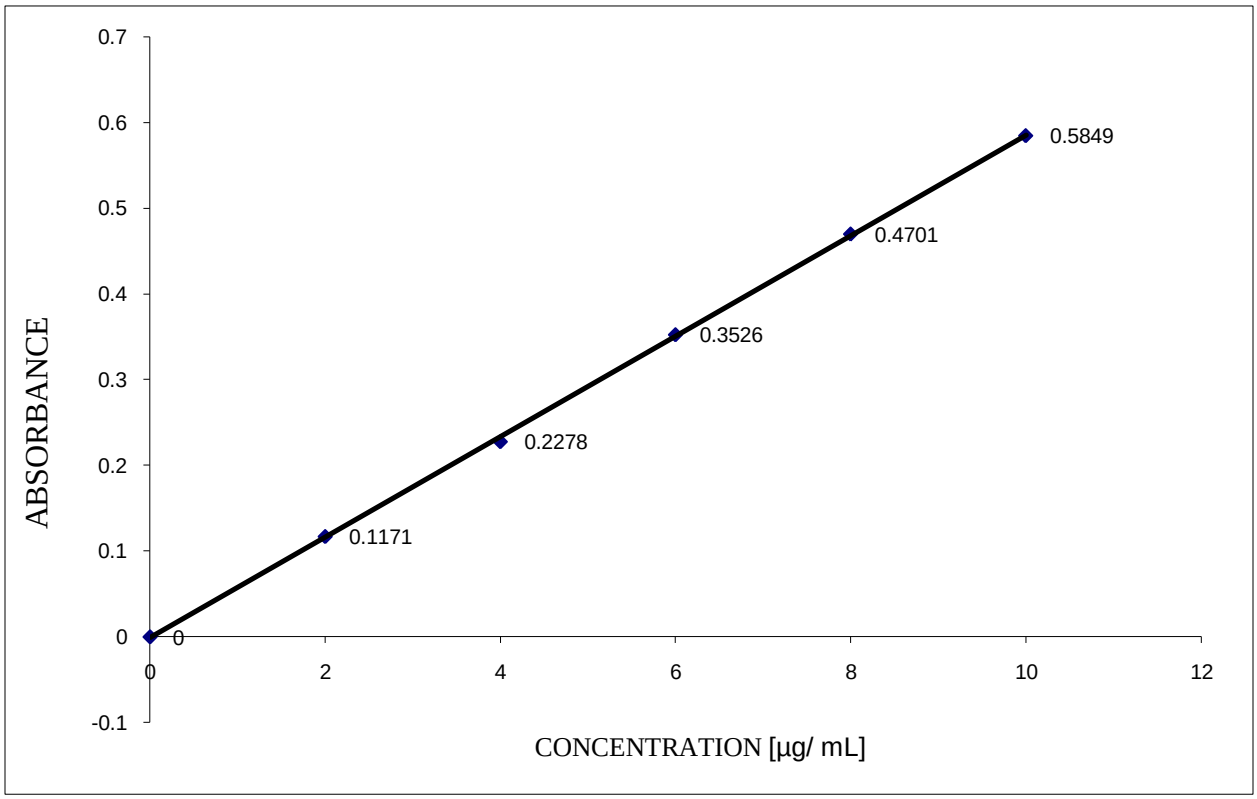


FIGURE – 9

CALIBRATION CURVE OF DIACEREIN IN

1 mL DIMETHYL SULPHOXIDE AND METHANOL AT 269 nm

(SIMULTANEOUS EQUATION METHOD)

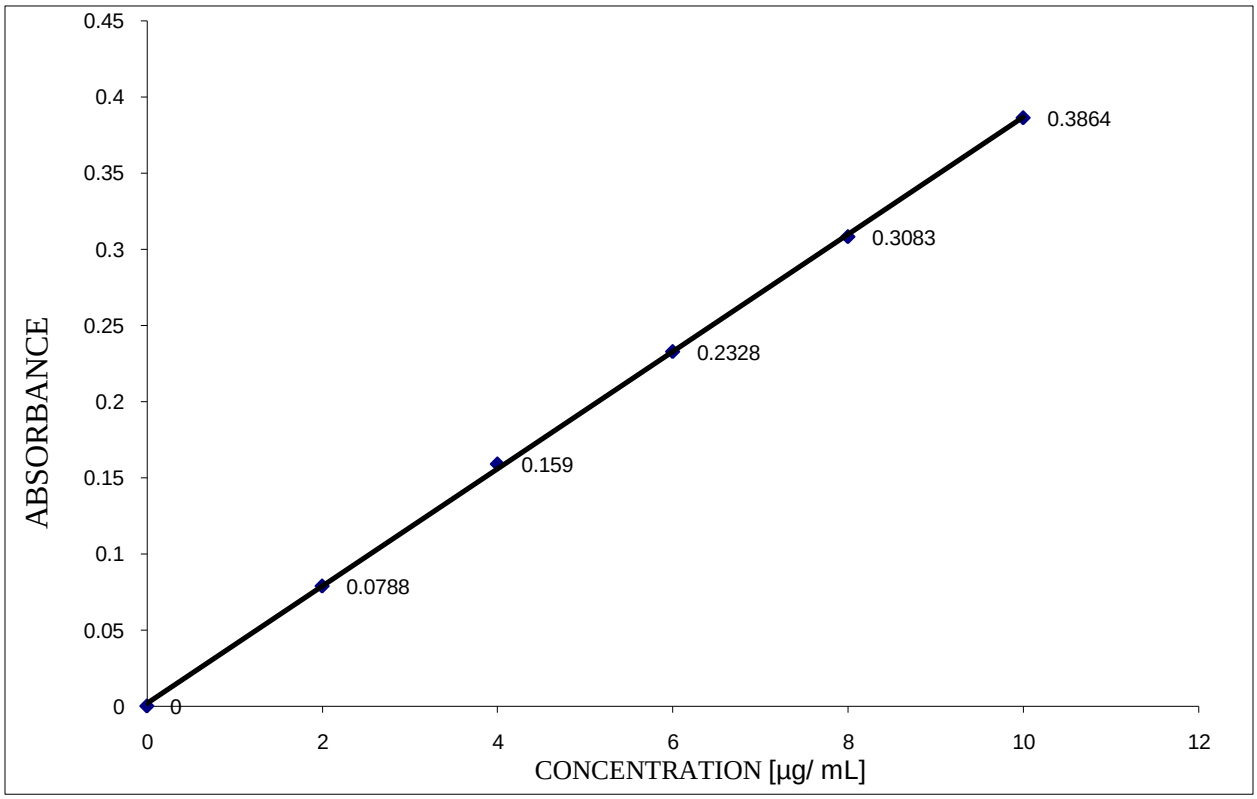


FIGURE – 10

CALIBRATION CURVE OF CELECOXIB IN

1 mL DIMETHYL SULPHOXIDE AND METHANOL AT 236 - 246 nm

(AREA UNDER CURVE)

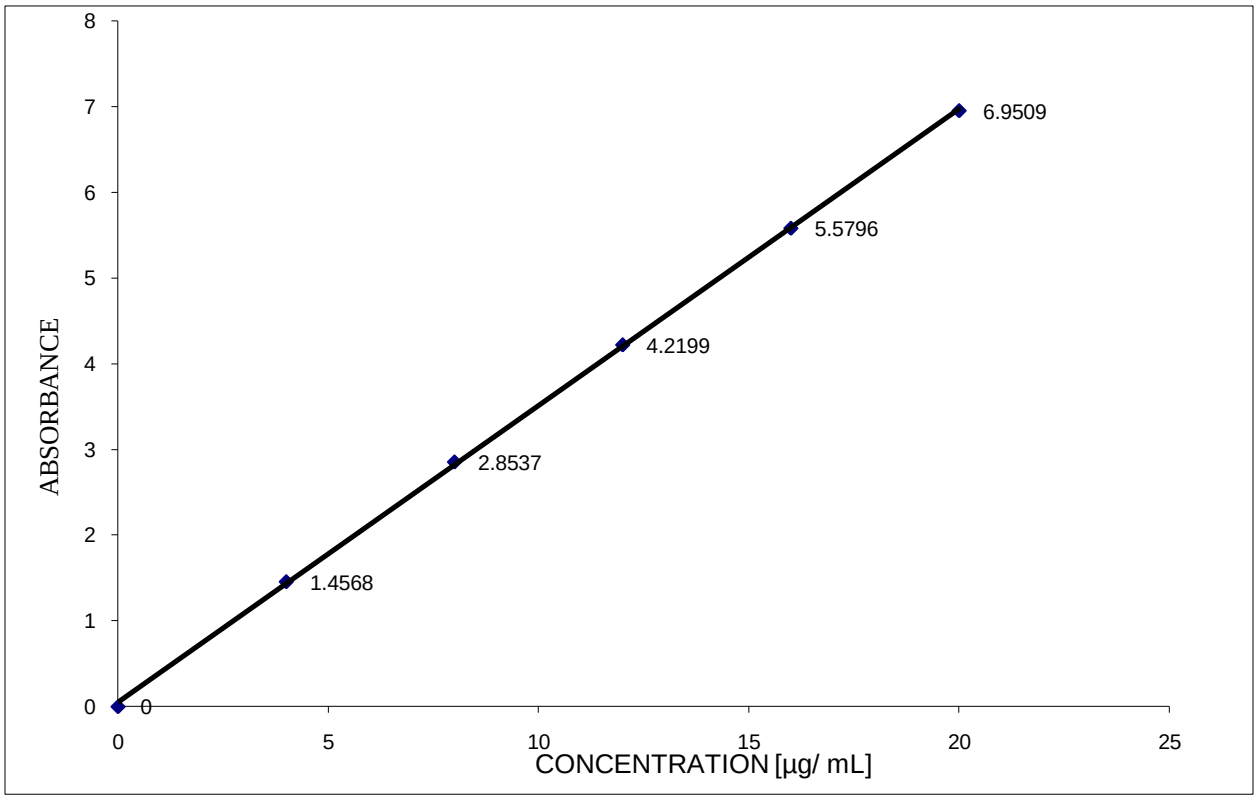


FIGURE – 11

CALIBRATION CURVE OF CELECOXIB IN

1 mL DIMETHYL SULPHOXIDE AND METHANOL AT 267 - 273 nm

(AREA UNDER CURVE)

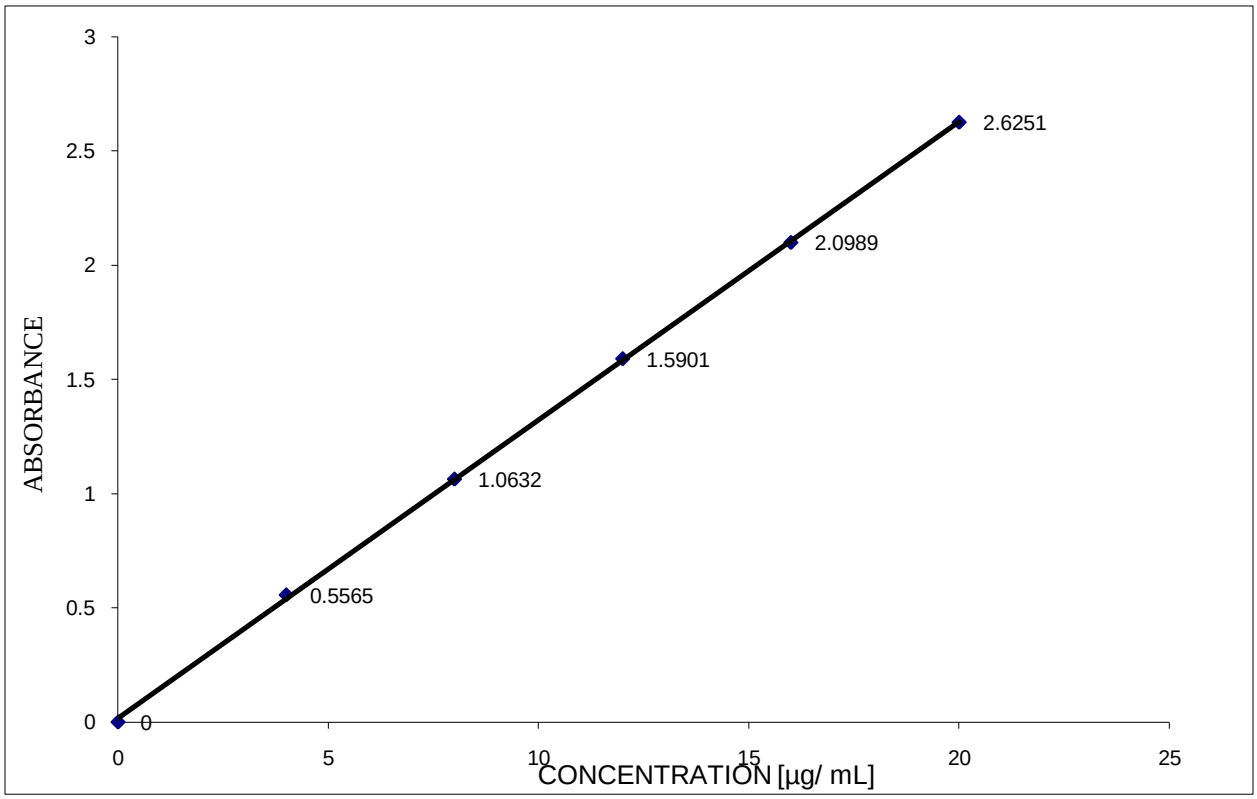


FIGURE – 12

CALIBRATION CURVE OF DIACEREIN IN

1 mL DIMETHYL SULPHOXIDE AND METHANOL AT 236 - 246 nm

(AREA UNDER CURVE)

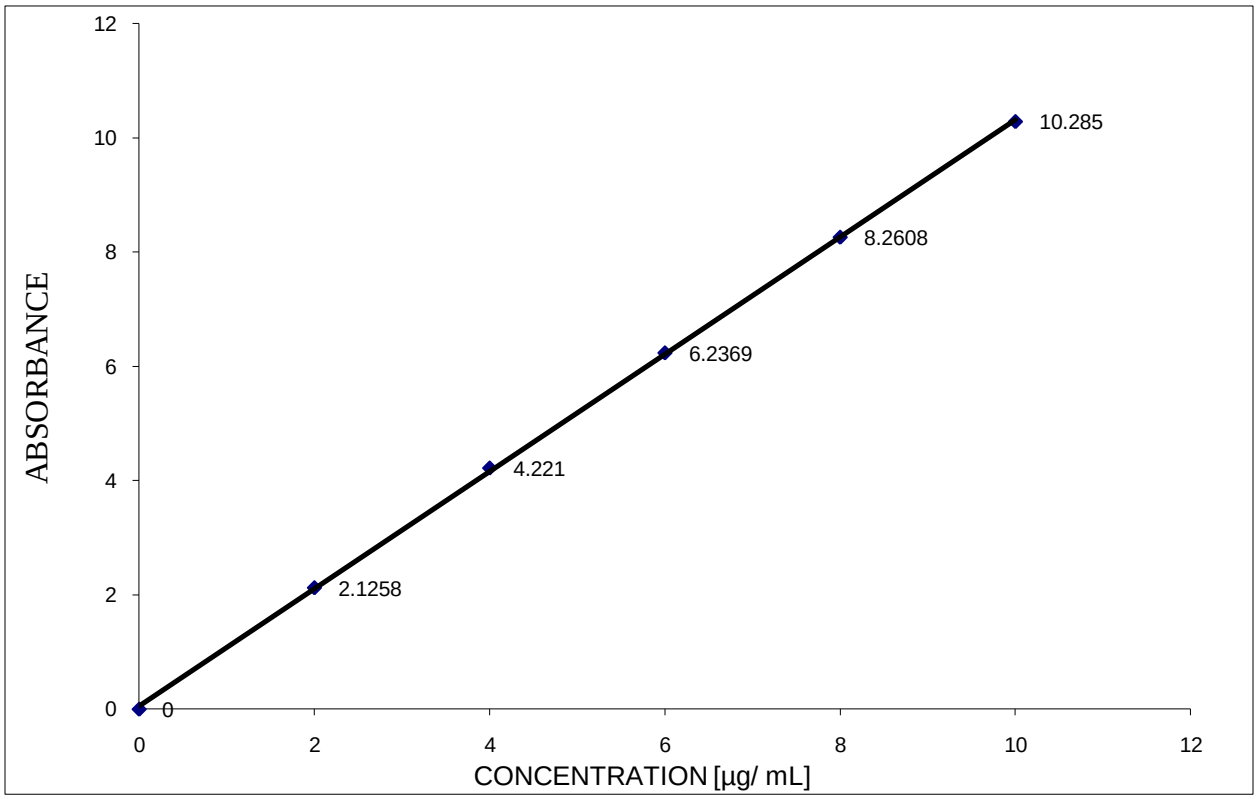


FIGURE – 13

CALIBRATION CURVE OF DIACEREIN IN

1 mL DIMETHYL SULPHOXIDE AND METHANOL AT 267 - 273 nm

(AREA UNDER CURVE)

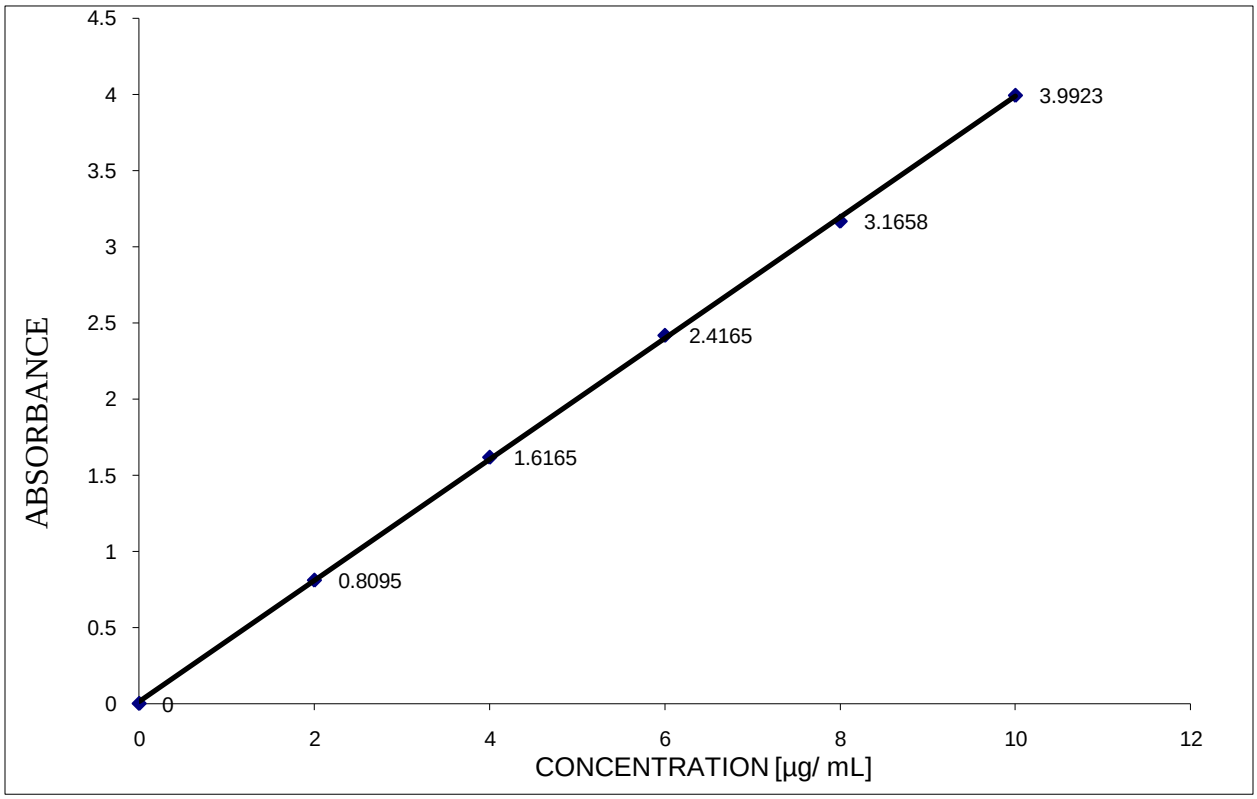
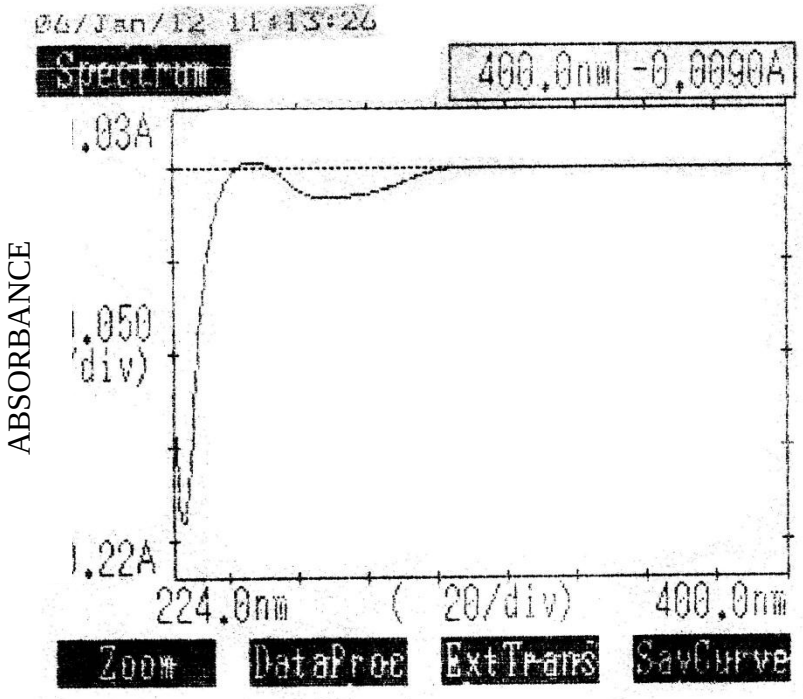


FIGURE – 14

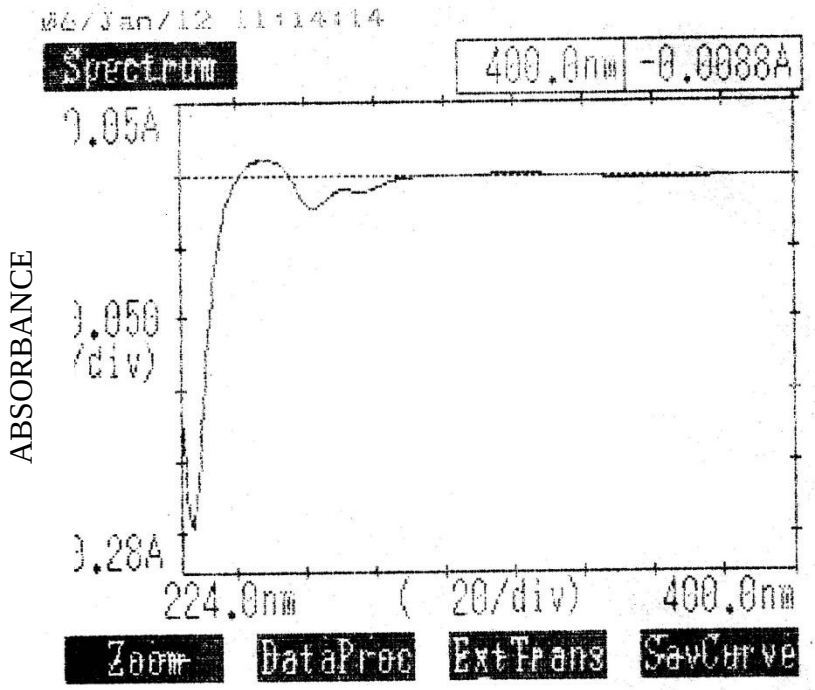
FIRST ORDER DERIVATIVE CURVE OF CELECOXIB AT 294 nm



Wavelength (nm)

FIGURE – 15

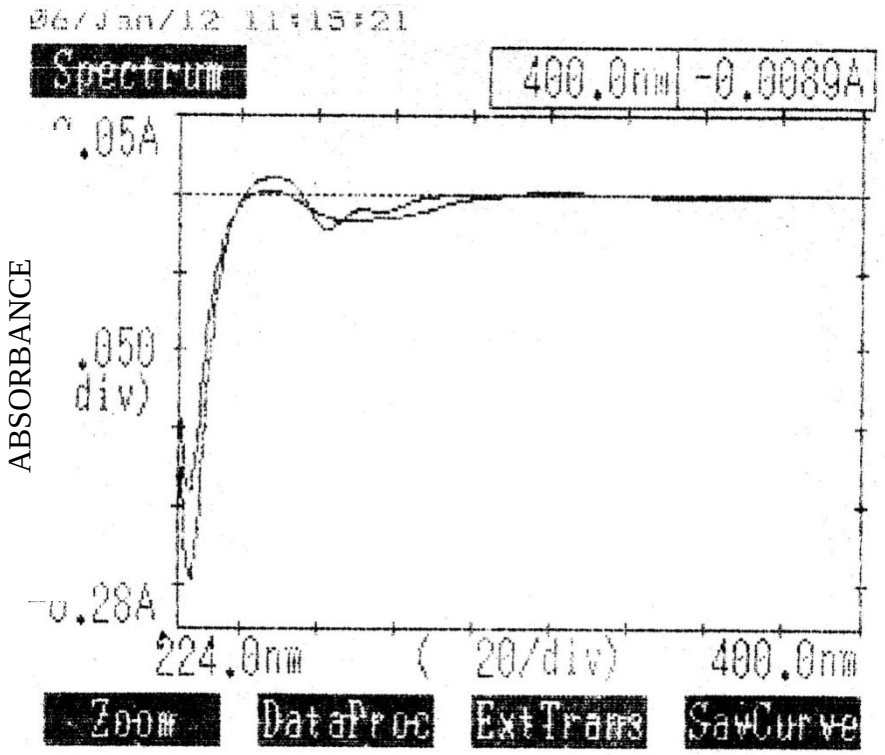
FIRST ORDER DERIVATIVE CURVE OF DIACEREIN AT 245 nm



Wavelength (nm)

FIGURE – 16

FIRST ORDER OVERLAID SPECTRUM OF CELECOXIB AND DIACEREIN



Wavelength (nm)

FIGURE – 17

CALIBRATION CURVE OF CELECOXIB IN

1 mL DIMETHYL SULPHOXIDE AND METHANOL AT 294 nm

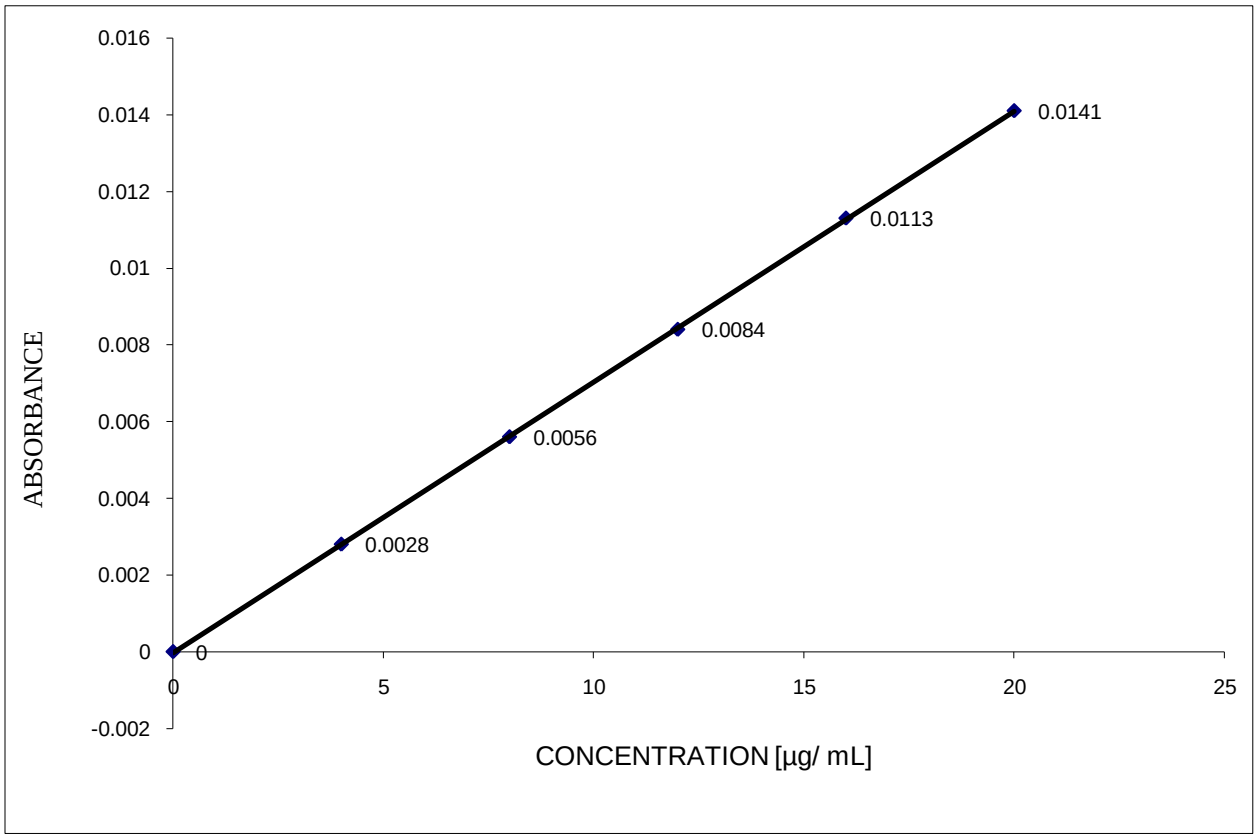


FIGURE – 18

CALIBRATION CURVE OF DIACEREIN IN

1 mL DIMETHYL SULPHOXIDE AND METHANOL AT 245 nm

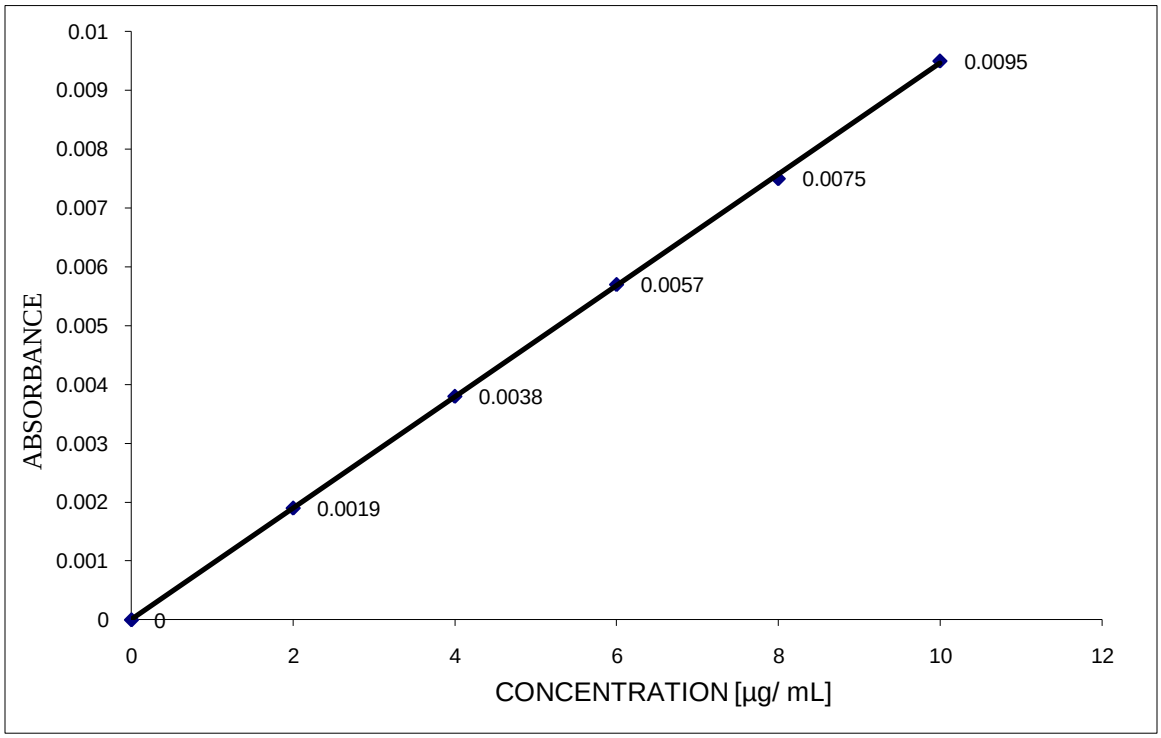
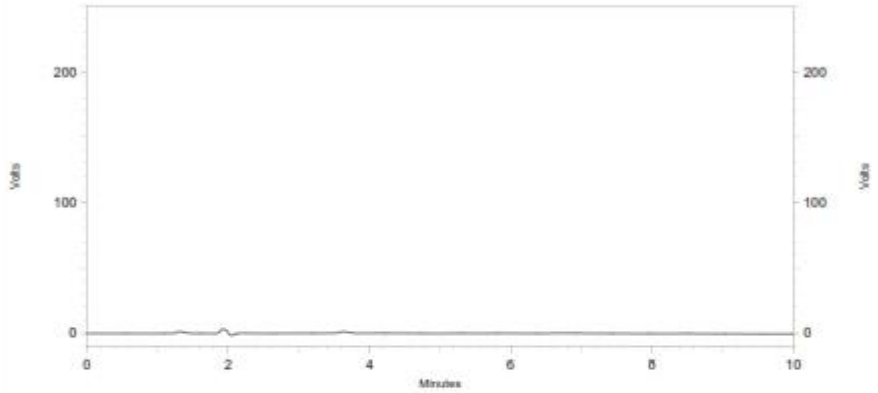


FIGURE – 19

BASE LINE CHROMATOGRAM USING METHANOL: WATER (85:15)

Area % Report

Description BLANK - METHANOL
Data File: D:\ChromQuest\PROJECTS\DATA\CELECOXCIB + DIACERIN\16.01.2012\001_BLANK.dat
Method: D:\ChromQuest\Method\CELECOX + DIACERIN.met
Acquired: 1/16/2012 8:19:49 PM
Wave Length 252 nm

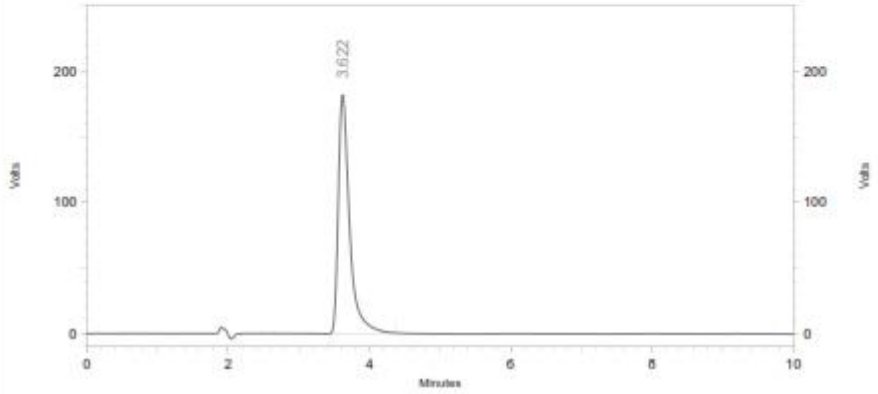


Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)

FIGURE – 20
INDIVIDUAL CHROMATOGRAM OF CELECOXIB USING METHANOL: WATER (85:15)

Area % Report

Description STD -CELECOXIB
Data File: D:\ChromQuest\PROJECTS\DATA\CELECOXCIB + DIACERIN\16.01.2012\TR054_STD.dat
Method: D:\ChromQuest\Method\CELECOX + DIACERIN.met
Acquired: 1/17/2012 10:41:17 AM
Wave Length 252 nm

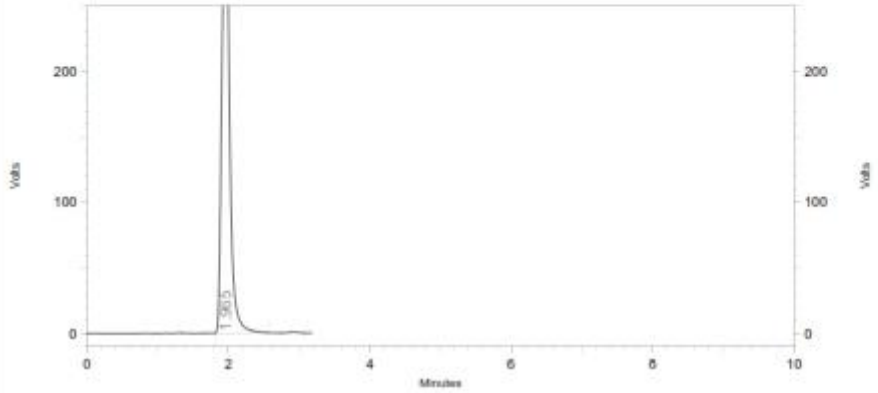


Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
CELECOXIB	3.622	2018275	100.00	193564	100.00	1.61	2653	0.00
Totals		2018275	100.00	193564	100.00			

FIGURE – 21
INDIVIDUAL CHROMATOGRAM OF DIACEREIN USING METHANOL: WATER (85:15)

Area % Report

Description STD DIACEREIN
Data File: D:\ChromQuest\PROJECTS\DATA\CELECOXCIB + DIACERIN\16.01.2012\TR009.dat
Method: D:\ChromQuest\Method\CELECOX + DIACERIN.met
Acquired: 1/16/2012 7:12:47 PM
Wave Length 252 nm



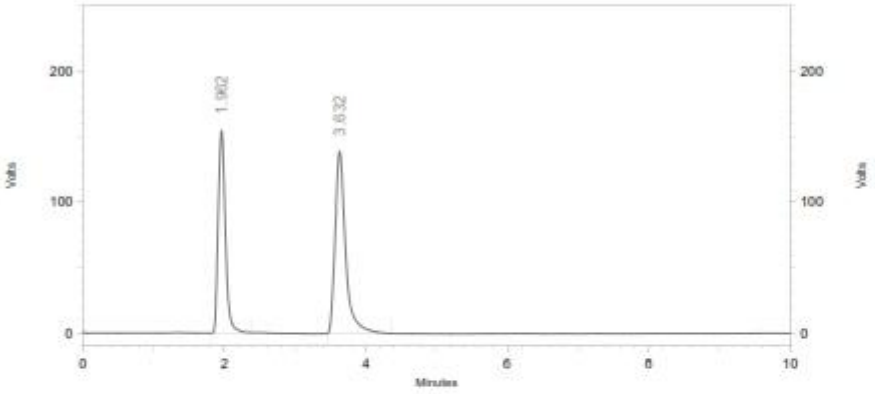
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
DIACEREIN	1.965	2649033	100.00	225461	100.00	1.27	1420	0.00
Totals		2649033	100.00	225461	100.00			

FIGURE – 22

LINEARTY CHROMATOGRAM OF CELECOXIB (70 mcg/mL) AND DIACEREIN (35 mcg/mL) USING METHANOL: WATER (85:15)

Area % Report

Description STD 70% CONCENTRATION- Diacerein (35mcg/mL) + Celecoxib (70mcg/mL)
Data File: D:\ChromQuest\PROJECTS\DATA\CELECOXCIB + DIACERIN\16.01.2012\007_LINEARITY-01.dat
Method: D:\ChromQuest\Method\CELECOX + DIACERIN.met
Acquired: 1/16/2012 9:29:38 PM
Wave Length 252 nm



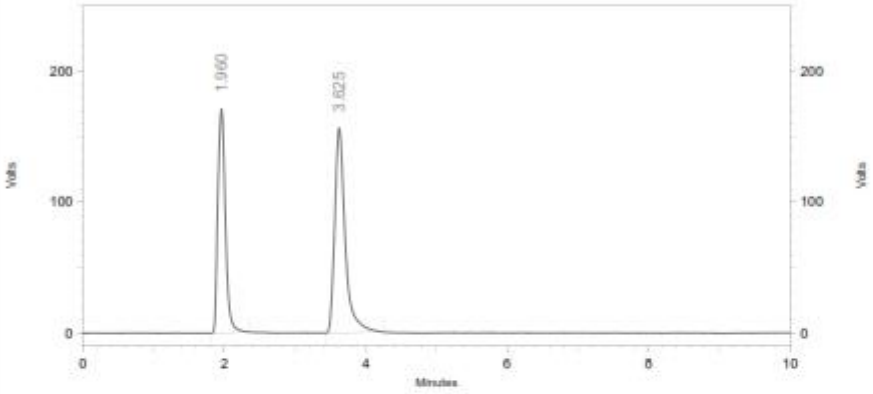
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
DIACEREIN	1.962	1094269	43.29	154653	52.65	1.27	1802	0.00
CELECOXIB	3.632	1433311	56.71	139107	47.35	1.54	3189	7.55
Totals		2527580	100.00	293760	100.00			

FIGURE – 23

LINEARTY CHROMATOGRAM OF CELECOXIB (80 mcg/mL) AND DIACEREIN (40 mcg/mL) USING METHANOL: WATER (85:15)

Area % Report

Description STD 80% CONCENTRATION- Diacerein (40mcg/mL) + Celecoxib (80mcg/mL)
Data File: D:\ChromQuest\PROJECTS\DATA\CELECOXCIB + DIACERIN\16.01.2012\008_LINEARITY-02.dat
Method: D:\ChromQuest\Method\CELECOX + DIACERIN.met
Acquired: 1/16/2012 9:41:15 PM
Wave Length 252 nm



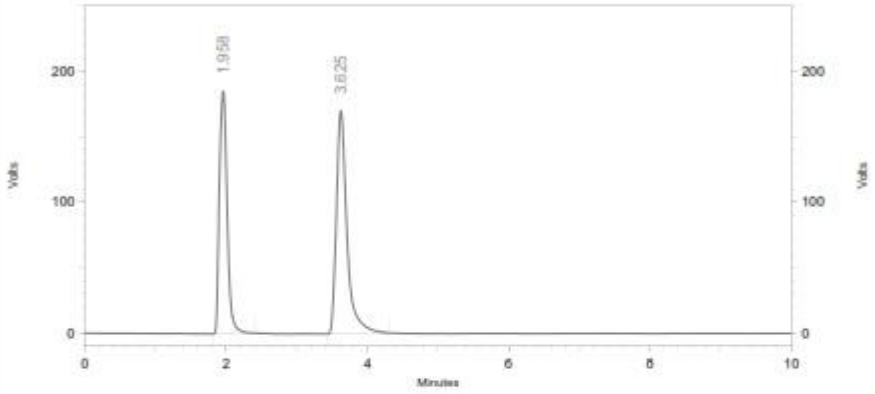
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
DIACEREIN	1.960	1268037	44.04	170839	52.23	1.26	1679	0.00
CELECOXIB	3.625	1611403	55.96	156264	47.77	1.56	3190	7.43
Totals		2879440	100.00	327103	100.00			

FIGURE – 24

LINEARTY CHROMATOGRAM OF CELECOXIB (90 mcg/mL) AND DIACEREIN (45 mcg/mL) USING METHANOL: WATER (85:15)

Area % Report

Description STD 90% CONCENTRATION- Diacerein (45mcg/mL) + Celecoxib (90mcg/mL)
Data File: D:\ChromQuest\PROJECTS\DATA\CELECOXCIB + DIACERIN\16.01.2012\009_LINEARITY-03.dat
Method: D:\ChromQuest\Method\CELECOX + DIACERIN.met
Acquired: 1/16/2012 9:52:51 PM
Wave Length 252 nm



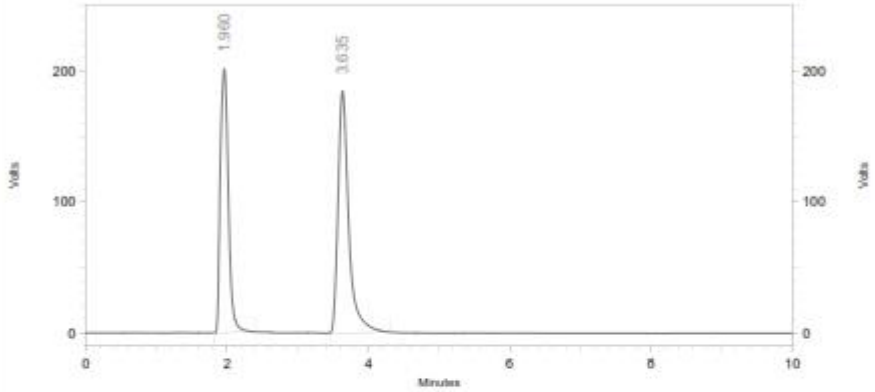
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
DIACEREIN	1.958	1416636	44.49	185320	52.15	1.26	1611	0.00
CELECOXIB	3.625	1767542	55.51	170036	47.85	1.58	3172	7.36
Totals		3184178	100.00	355356	100.00			

FIGURE – 25

LINEARTY CHROMATOGRAM OF CELECOXIB (100 mcg/mL) AND DIACEREIN (50 mcg/mL) USING METHANOL: WATER (85:15)

Area % Report

Description STD 100% CONCENTRATION- Diacerein (50mcg/mL) + Celecoxib (100mcg/mL)
Data File: D:\ChromQuest\PROJECTS\DATA\CELECOXCIB + DIACERIN\16.01.2012\010_LINEARITY-04.dat
Method: D:\ChromQuest\Method\CELECOX + DIACERIN.met
Acquired: 1/16/2012 10:04:27 PM
Wave Length 252 nm



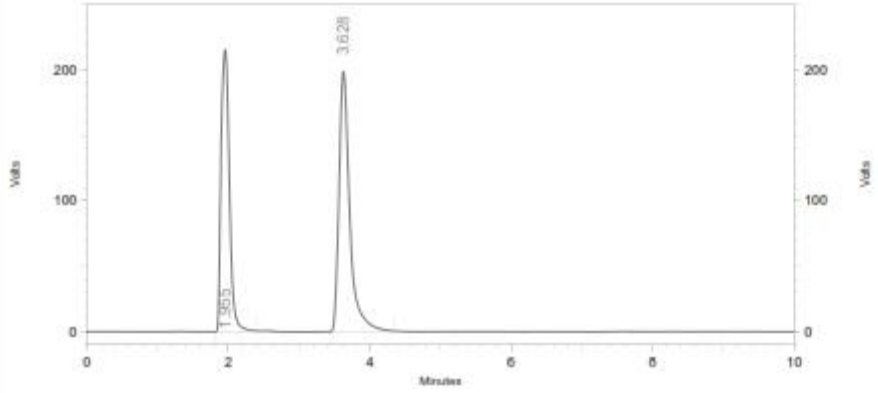
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
DIACEREIN	1.960	1594745	45.10	201365	52.15	1.27	1551	0.00
CELECOXIB	3.635	1941048	54.90	184745	47.85	1.59	3139	7.30
Totals		3535793	100.00	386110	100.00			

FIGURE – 26

LINEARTY CHROMATOGRAM OF CELECOXIB (110 mcg/mL) AND DIACEREIN (55 mcg/mL) USING METHANOL: WATER (85:15)

Area % Report

Description STD 110% CONCENTRATION- Diacerein (55mcg/mL) + Celecoxib (110mcg/mL)
Data File: D:\ChromQuest\PROJECTS\DATA\CELECOXCIB + DIACERIN\16.01.2012\011_LINEARITY-05.dat
Method: D:\ChromQuest\Method\CELECOX + DIACERIN.met
Acquired: 1/16/2012 10:16:07 PM
Wave Length 252 nm



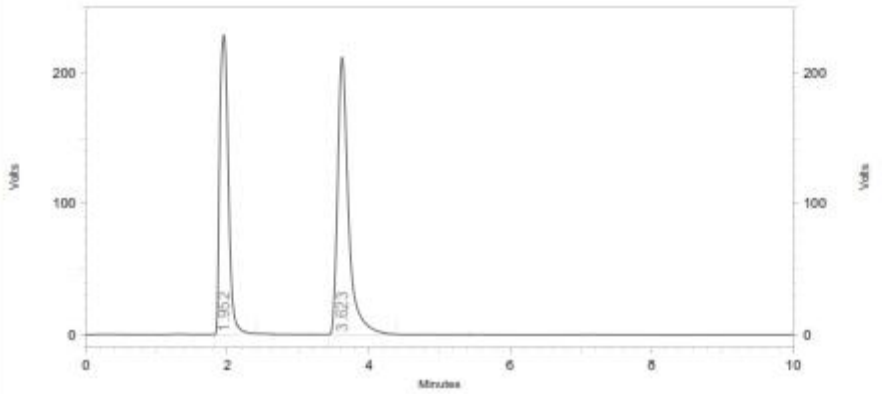
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
DIACEREIN	1.955	1763244	45.65	215377	52.02	1.29	1472	0.00
CELECOXIB	3.628	2099425	54.35	198638	47.98	1.63	3096	7.20
Totals		3862669	100.00	414015	100.00			

FIGURE – 27

LINEARTY CHROMATOGRAM OF CELECOXIB (120 mcg/mL) AND DIACEREIN (60 mcg/mL) USING METHANOL: WATER (85:15)

Area % Report

Description STD 120% CONCENTRATION- Diacerein (60mcg/mL) + Celecoxib (120mcg/mL)
Data File: D:\ChromQuest\PROJECTS\DATA\CELECOXCIB + DIACERIN\16.01.2012\012_LINEARITY-06.dat
Method: D:\ChromQuest\Method\CELECOX + DIACERIN.met
Acquired: 1/16/2012 10:27:46 PM
Wave Length 252 nm



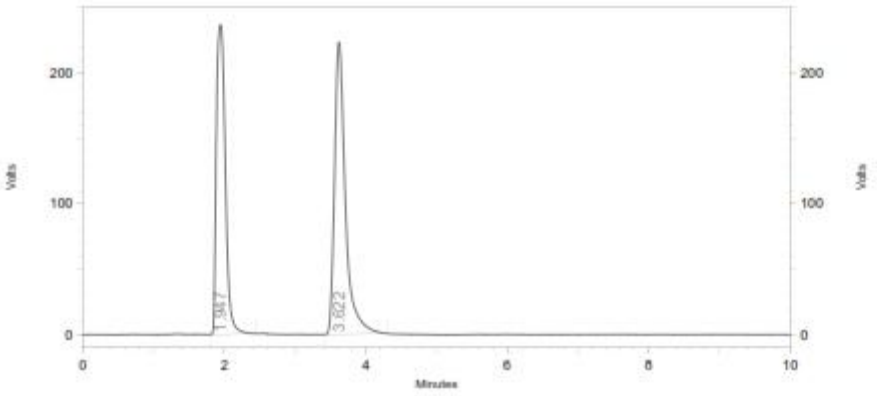
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
DIACEREIN	1.952	1936524	46.08	228620	51.90	1.31	1414	0.00
CELECOXIB	3.623	2265888	53.92	211882	48.10	1.65	3047	7.11
Totals		4202412	100.00	440502	100.00			

FIGURE – 28

LINEARTY CHROMATOGRAM OF CELECOXIB (130 mcg/mL) AND DIACEREIN (65 mcg/mL) USING METHANOL: WATER (85:15)

Area % Report

Description STD 130% CONCENTRATION- Diacerein (65mcg/mL) + Celecoxib (130mcg/mL)
Data File: D:\ChromQuest\PROJECTS\DATA\CELECOXCIB + DIACERIN\16.01.2012\013_LINEARITY-07.dat
Method: D:\ChromQuest\Method\CELECOX + DIACERIN.met
Acquired: 1/16/2012 10:39:25 PM
Wave Length 252 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
DIACEREIN	1.947	2085118	46.08	236448	51.44	1.35	1333	0.00
CELECOXIB	3.622	2440289	53.92	223178	48.56	1.66	3028	7.03
Totals		4525407	100.00	459626	100.00			

FIGURE – 29
CALIBRATION CURVE OF CELECOXIB

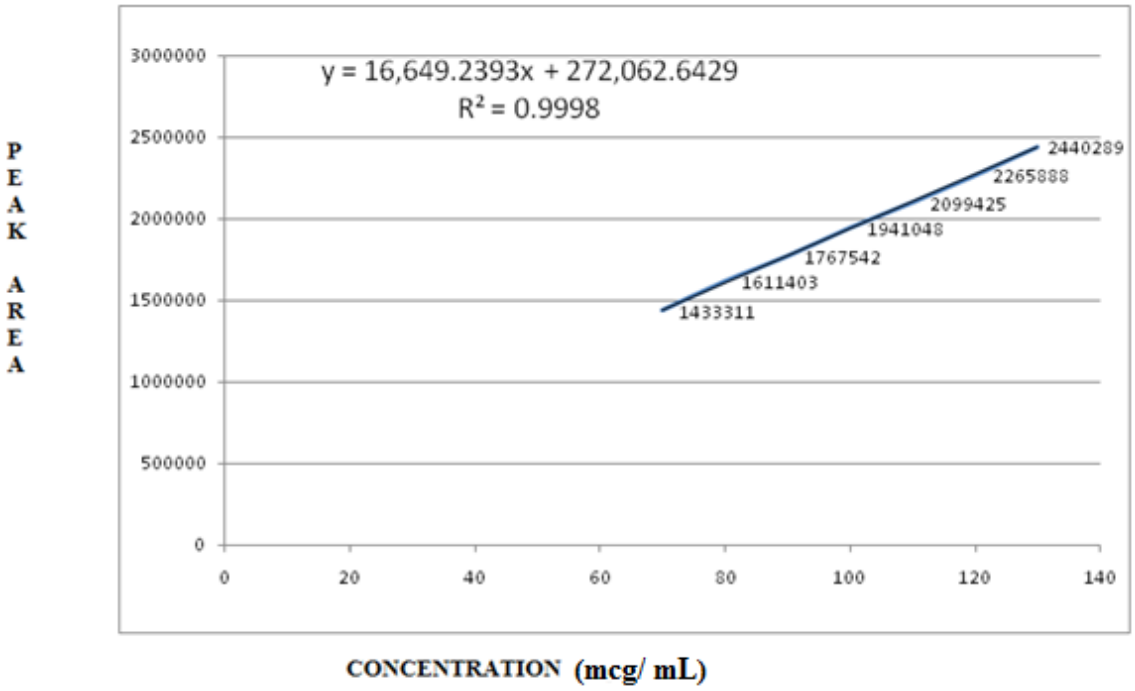


FIGURE – 30

CALIBRATION CURVE OF DIACEREIN

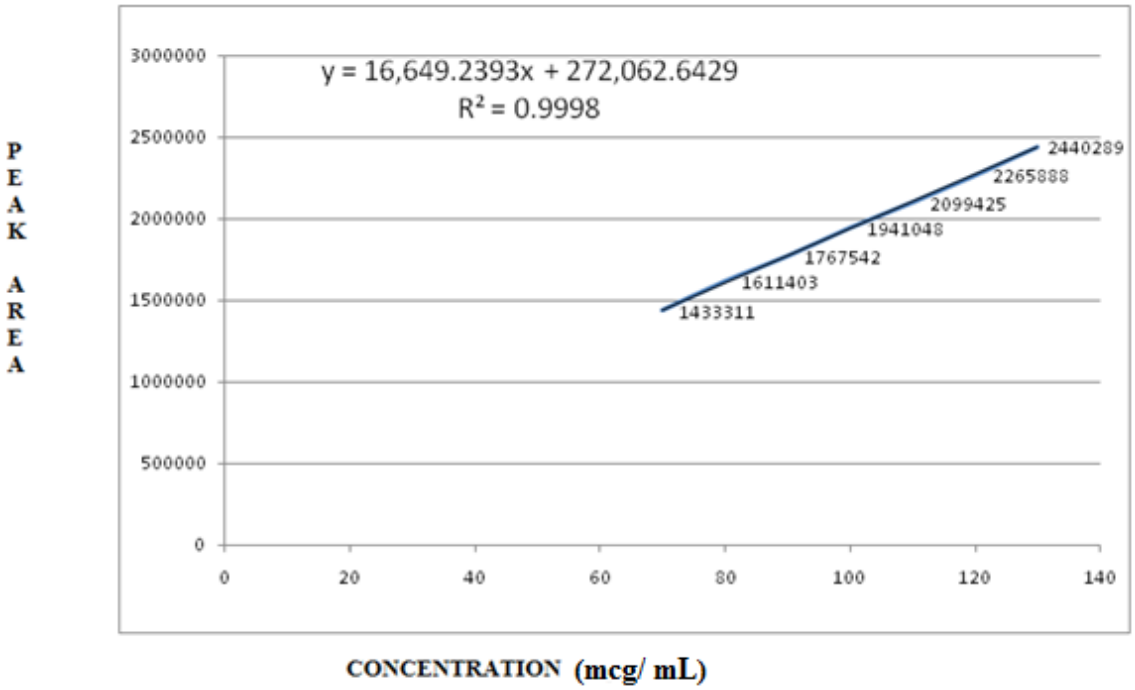
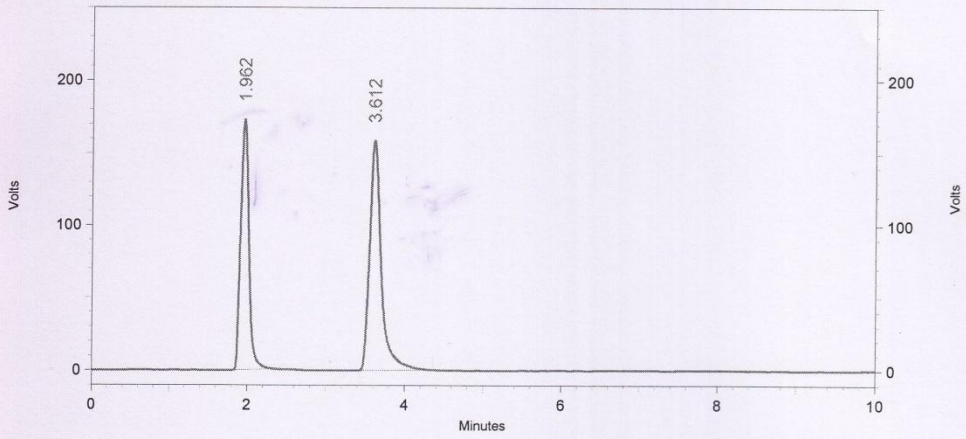


FIGURE – 31

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (OSTIGARD®) IN LOW LEVEL CONCENTRATION-80% (1)

Area % Report

Description SPL- 01_-OSTIGARD 100 - LOW LEVEL CONCENTRATION - 80%
Data File: D:\ChromQuest\PROJECTS\DATA\CELECOXCIB + DIACERIN\16.01.2012\014_PA_L1.dat
Method: D:\ChromQuest\Method\CELECOX + DIACERIN.met
Acquired: 1/16/2012 10:51:01 PM
Wave Length 252 nm



Name	Retention Time	Area	Area %
DIACEREIN	1.962	1252089	44.99
CELECOXIB	3.612	1531185	55.01

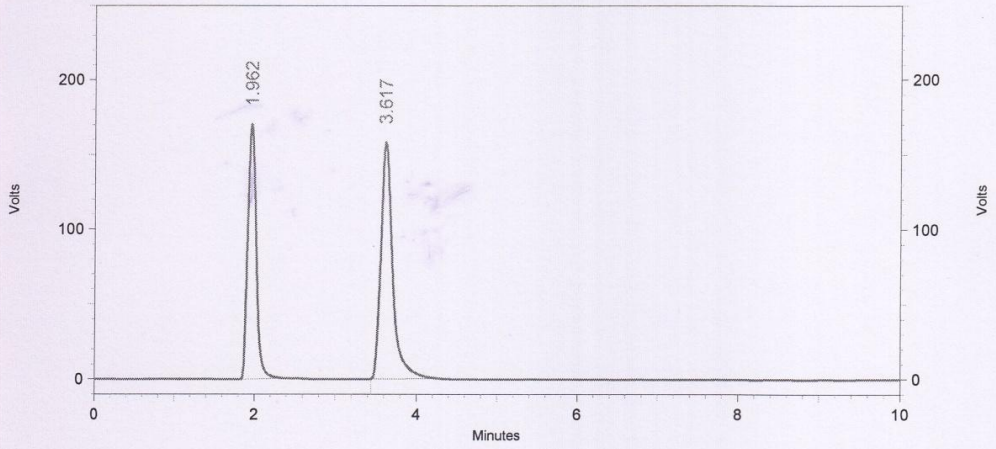
Totals			
		2783274	100.00

FIGURE –32

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (OSTIGARD®) IN LOW LEVEL CONCENTRATION-80% (2)

Area % Report

Description SPL- 02_-OSTIGARD 100 - LOW LEVEL CONCENTRATION - 80%
Data File: D:\ChromQuest\PROJECTS\DATA\CELECOXCIB + DIACERIN\16.01.2012\015_PA_L2.dat
Method: D:\ChromQuest\Method\CELECOX + DIACERIN.met
Acquired: 1/16/2012 11:02:39 PM
Wave Length 252 nm



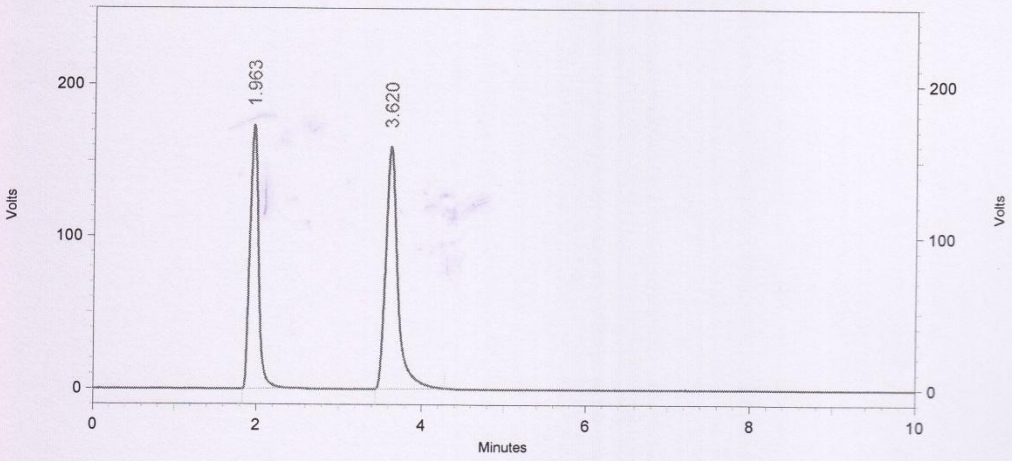
Name	Retention Time	Area	Area %
DIACEREIN	1.962	1249027	44.88
CELECOXIB	3.617	1533811	55.12
Totals		2782838	100.00

FIGURE – 33

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (OSTIGARD®) IN LOW LEVEL CONCENTRATION-80% (3)

Area % Report

Description SPL- 03_-OSTIGARD 100 - LOW LEVEL CONCENTRATION - 80%
Data File: D:\ChromQuest\PROJECTS\DATA\CELECOXCIB + DIACERIN\16.01.2012\016_PA_L3.dat
Method: D:\ChromQuest\Method\CELECOX + DIACERIN.met
Acquired: 1/16/2012 11:14:15 PM
Wave Length 252 nm

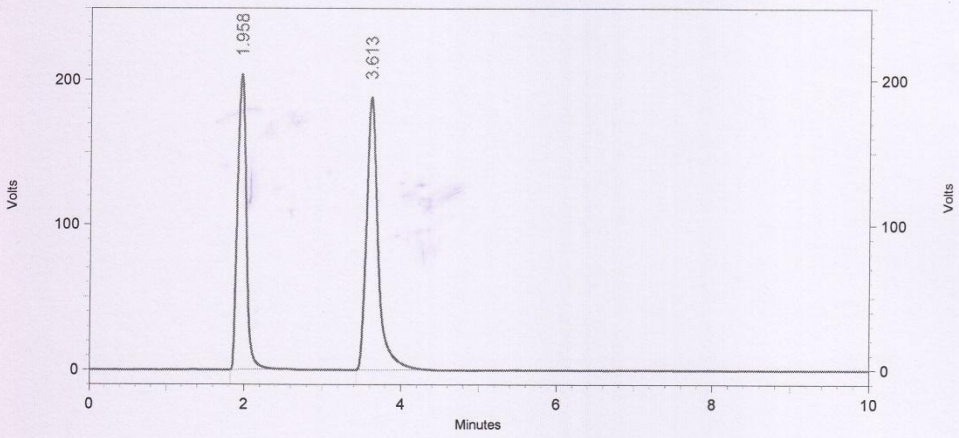


Name	Retention Time	Area	Area %
DIACEREIN	1.963	1256470	44.94
CELECOXIB	3.620	1539259	55.06
Totals		2795729	100.00

FIGURE – 34
CHROMATOGRAM FOR ANALYSIS OF FORMULATION (OSTIGARD®) IN MID LEVEL CONCENTRATION-100% (1)

Area % Report

Description SPL- 01_-OSTIGARD 100 - MIDDLE LEVEL CONCENTRATION - 100%
Data File: D:\ChromQuest\PROJECTS\DATA\CELECOXCIB + DIACERIN\16.01.2012\017_PA_M1.dat
Method: D:\ChromQuest\Method\CELECOX + DIACERIN.met
Acquired: 1/16/2012 11:25:53 PM
Wave Length 252 nm



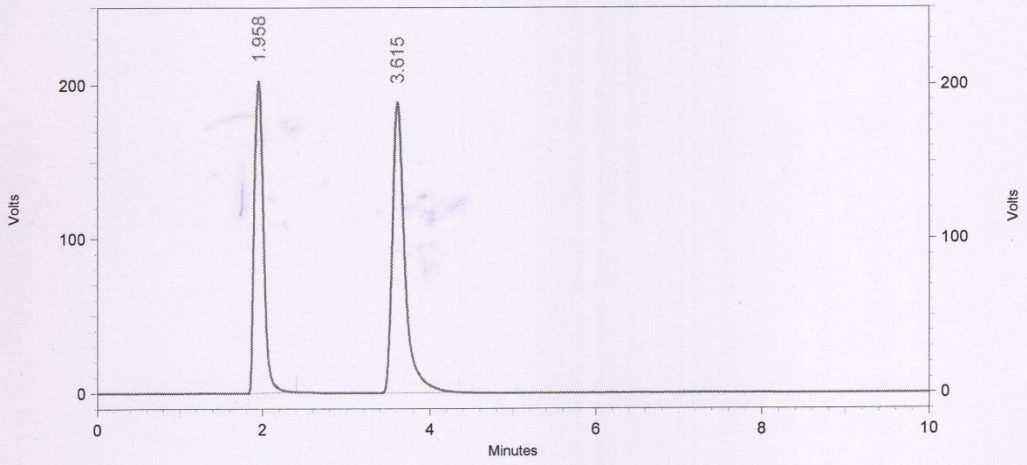
Name	Retention Time	Area	Area %
DIACEREIN	1.958	1584788	44.96
CELECOXIB	3.613	1940178	55.04
Totals		3524966	100.00

FIGURE – 35

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (OSTIGARD® IN MID LEVEL CONCENTRATION-100% (2)

Area % Report

Description SPL- 02_-OSTIGARD 100 - MIDDLE LEVEL CONCENTRATION - 100%
Data File: D:\ChromQuest\PROJECTS\DATA\CELECOXCIB + DIACERIN\16.01.2012\018_PA_M2.dat
Method: D:\ChromQuest\Method\CELECOX + DIACERIN.met
Acquired: 1/16/2012 11:37:31 PM
Wave Length 252 nm



Name	Retention Time	Area	Area %
DIACEREIN	1.958	1592831	45.00
CELECOXIB	3.615	1946856	55.00

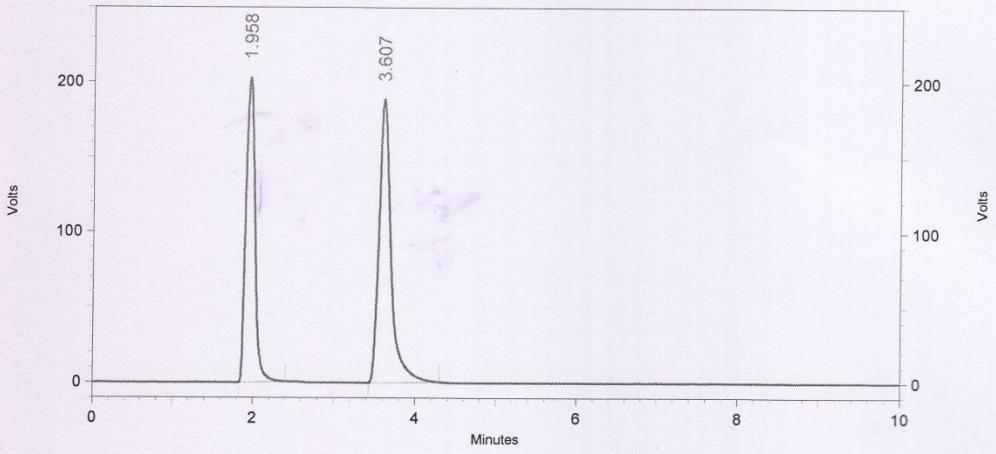
Totals			
		3539687	100.00

FIGURE – 36

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (OSTIGARD®) IN MID LEVEL CONCENTRATION-100% (3)

Area % Report

Description SPL- 03_-OSTIGARD 100 - MIDDLE LEVEL CONCENTRATION - 100%
Data File: D:\ChromQuest\PROJECTS\DATA\CELECOXCIB + DIACERIN\16.01.2012\019_PA_M3.dat
Method: D:\ChromQuest\Method\CELECOX + DIACERIN.met
Acquired: 1/16/2012 11:49:08 PM
Wave Length 252 nm



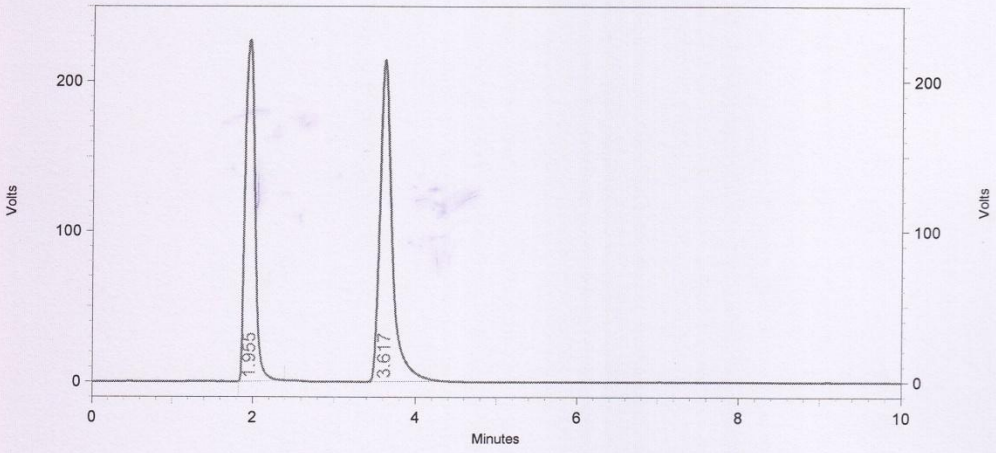
Name	Retention Time	Area	Area %
DIACEREIN	1.958	1583273	44.98
CELECOXIB	3.607	1936683	55.02
Totals		3519956	100.00

FIGURE – 37

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (OSTIGARD®) IN HIGH LEVEL CONCENTRATION-120% (1)

Area % Report

Description SPL- 01_-OSTIGARD 100 - HIGH LEVEL CONCENTRATION - 120%
Data File: D:\ChromQuest\PROJECTS\DATA\CELECOXCIB + DIACERIN\16.01.2012\020_PA_H1.dat
Method: D:\ChromQuest\Method\CELECOX + DIACERIN.met
Acquired: 1/17/2012 12:00:46 AM
Wave Length 252 nm



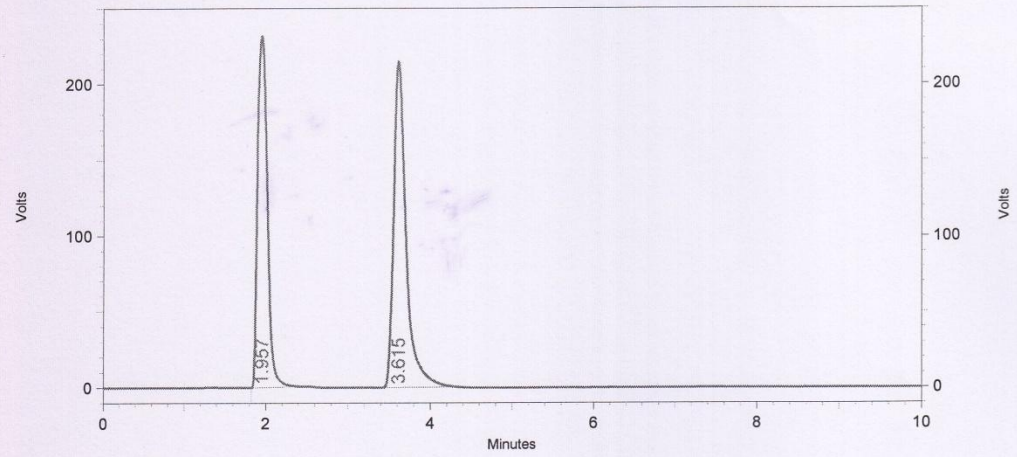
Name	Retention Time	Area	Area %
DIACEREIN	1.955	1901045	46.92
CELECOXIB	3.617	2331389	55.08
Totals		4232434	100.00

FIGURE – 38

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (OSTIGARD®) IN HIGH LEVEL CONCENTRATION-120% (2)

Area % Report

Description SPL- 02_-OSTIGARD 100 - HIGH LEVEL CONCENTRATION - 120%
Data File: D:\ChromQuest\PROJECTS\DATA\CELECOXCIB + DIACERIN\16.01.2012\021_PA_H2.dat
Method: D:\ChromQuest\Method\CELECOX + DIACERIN.met
Acquired: 1/17/2012 12:12:23 AM
Wave Length 252 nm



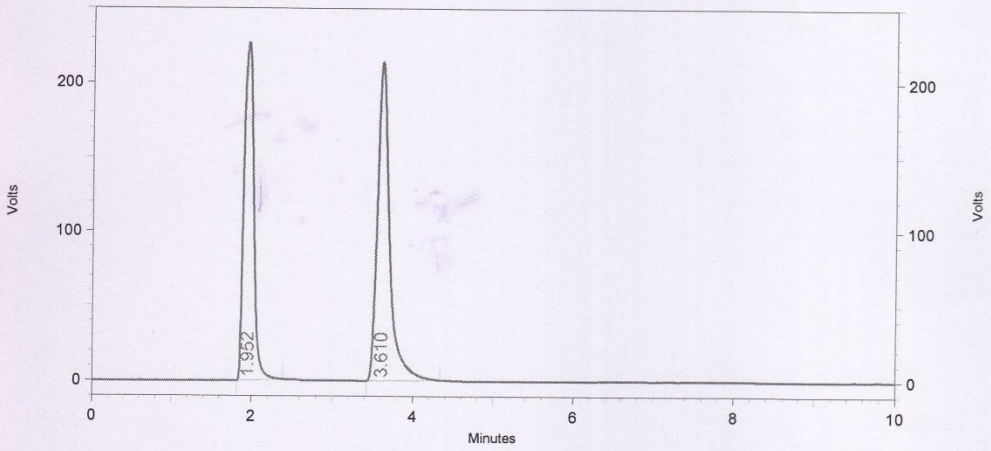
Name	Retention Time	Area	Area %
DIACEREIN	1.957	1919343	45.01
CELECOXIB	3.615	2344799	54.99
Totals		4264142	100.00

FIGURE – 39

CHROMATOGRAM FOR ANALYSIS OF FORMULATION (OSTIGARD®) IN HIGH LEVEL CONCENTRATION-120% (3)

Area % Report

Description SPL- 03_-OSTIGARD 100 - HIGH LEVEL CONCENTRATION - 120%
Data File: D:\ChromQuest\PROJECTS\DATA\CELECOXCIB + DIACERIN\16.01.2012\022_PA_H3.dat
Method: D:\ChromQuest\Method\CELECOX + DIACERIN.met
Acquired: 1/17/2012 12:24:03 AM
Wave Length 252 nm



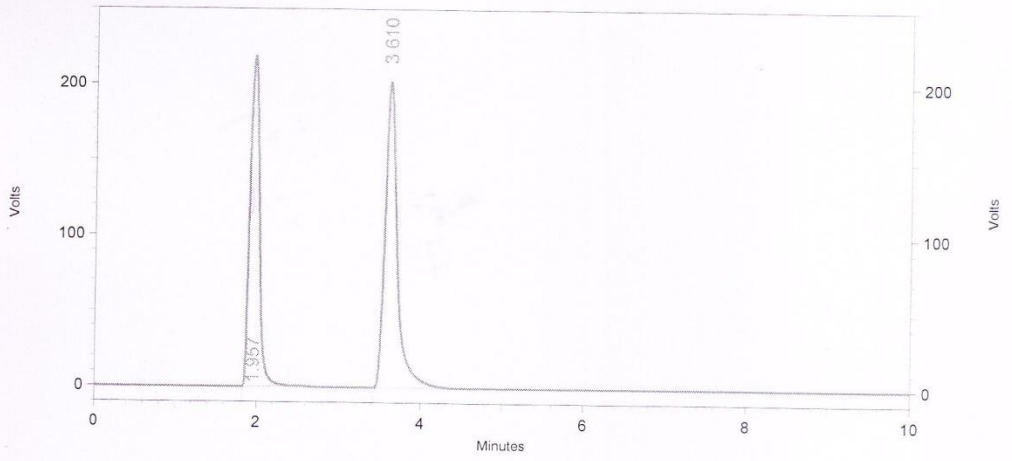
Name	Retention Time	Area	Area %
DIACEREIN	1.952	1905941	44.98
CELECOXIB	3.610	2331548	55.02
Totals		4237489	100.00

FIGURE – 40

CHROMATOGRAM FOR 110% RECOVERY OF FORMULATION (OSTIGARD®)

Area % Report

Description SPL-RECOVERY- 01_OSTIGARD 100 - STANDARD 10%
Data File: D:\ChromQuest\PROJECTS\DATA\CELECOXCIB + DIACERIN\16.01.2012\023_RECOVERY-110%.dat
Method: D:\ChromQuest\Method\CELECOX + DIACERIN.met
Acquired: 1/17/2012 12:35:43 AM
Wave Length 252 nm



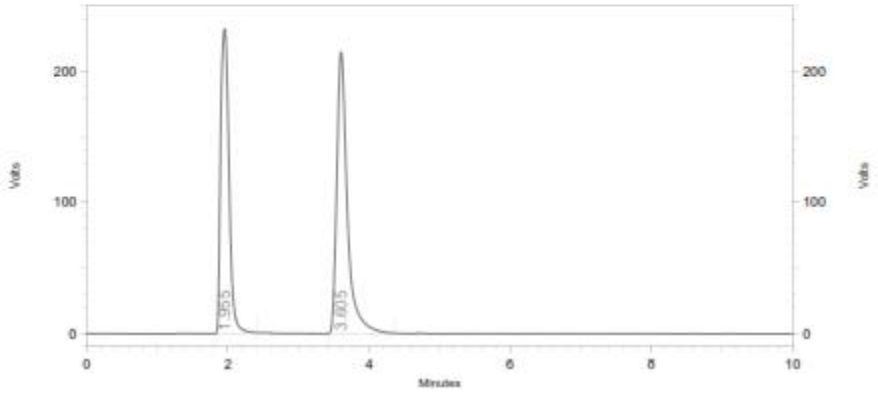
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
DIACEREIN	1.957	1745243	44.95	218730	51.98	1.28	1526	0.00
CELECOXIB	3.610	2137662	55.05	202065	48.02	1.64	3239	7.28
Totals		3882905	100.00	420795	100.00			

FIGURE – 41

CHROMATOGRAM FOR 120% RECOVERY OF FORMULATION (OSTIGARD®)

Area % Report

Description SPL-RECOVERY- 02_OSTIGARD 100 - STANDARD 20%
Data File: D:\ChromQuest\PROJECTS\DATA\CELECOXCIB + DIACERIN\16.01.2012\024_RECOVERY-120%.dat
Method: D:\ChromQuest\Method\CELECOX + DIACERIN.met
Acquired: 1/17/2012 12:47:24 AM
Wave Length 252 nm



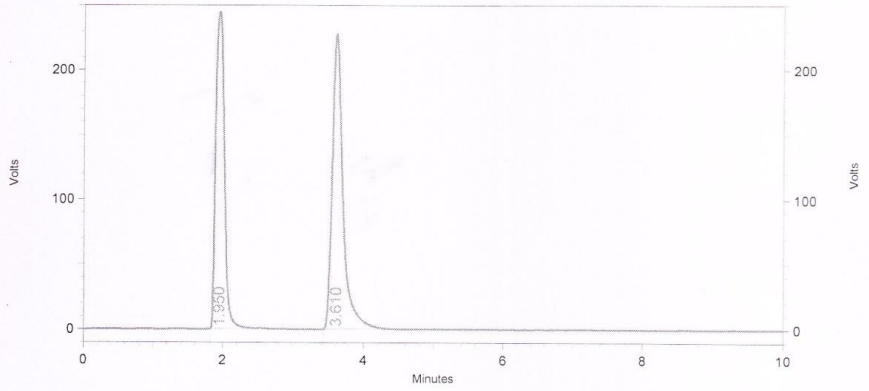
Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
DIACEREIN	1.955	1904481	44.93	232451	51.98	1.31	1477	0.00
CELECOXIB	3.605	2334281	55.07	214753	48.02	1.66	3215	7.21
Totals		4238762	100.00	447204	100.00			

FIGURE – 42

CHROMATOGRAM FOR 130% RECOVERY OF FORMULATION (OSTIGARD®)

Area % Report

Description SPL-RECOVERY- 03_OSTIGARD 100 - STANDARD 30%
Data File: D:\ChromQuest\PROJECTS\DATA\CELECOXCIB + DIACERIN\16.01.2012\025_RECOVERY-130%.dat
Method: D:\ChromQuest\Method\CELECOX + DIACERIN.met
Acquired: 1/17/2012 12:59:02 AM
Wave Length 252 nm



Name	Retention Time	Area	Area %	Height	Height Percent	Asymmetry	Theoretical plates (USP)	Resolution (USP)
DIACEREIN	1.950	2062351	44.95	244387	51.79	1.34	1411	0.00
CELECOXIB	3.610	2526071	55.05	227539	48.21	1.67	3163	7.15
Totals		4588422	100.00	471926	100.00			

TABLES

TABLE-1

SOLUBILITY PROFILE OF CELECOXIB AND DIACEREIN

S. No.	SOLVENTS	CELECOXIB	DIACEREIN
1.	Acetonitrile	Soluble	Slightly Soluble
2.	Acetone	Soluble	Slightly Soluble
3.	Butanol	Soluble	Very slightly soluble
4.	Benzene	Soluble	Very slightly soluble
5.	Carbon tetra chloride	Soluble	Very slightly soluble
6.	Chloroform	Soluble	Slightly Soluble
7.	Dimethyl formamide	Soluble	Sparingly Soluble
8.	Dimeththylsulphoxide (DMSO)	Soluble	Soluble
9.	Distilled water	Insoluble	Insoluble
10.	Ethanol	Soluble	Slightly Soluble
11.	Ethyl acetate	Soluble	Soluble
12.	HCl (0.1 N)	Soluble	Insoluble
13.	Methanol	Soluble	Slightly Soluble
14.	NaOH (0.1 N)	In Soluble	Sparingly Insoluble
15.	Toluene	Soluble	Very slightly soluble

TABLE-2

OPTICAL PARAMETERS OF CELECOXIB AND DIACEREIN BY SIMULTANEOUS EQUATION METHOD

PARAMETERS	CELECOXIB	DIACEREIN
λ_{max}(nm)	236	269
Beers law limit (µg/ mL)	4-20	2-10
Sandell's sensitivity (µg/cm^2/0.001 A.U)	0.032816	0.025325
Molar absorptivity (L mol^{-1} cm^{-1})	16918.74	14276.72
Correlation coefficient (r)	0.99996	0.99997
Regression equation (y = mx + c)	Y = 0.062922 X + 0.00381	Y = 0.038448 X + 0.002269
Slope(m)	0.062922	0.038448
Intercept(c)	0.00381	0.002269
LOD (µg /mL)	0.091034519	0.0034165157
LOQ (µg/ mL)	0.275862176	0.103530778
Standard error of mean of Regression line	0.000278	0.000095918

TABLE-3

ASSAY OF COMMERCIAL FORMULATION BY UV- SPECTROSCOPY

(SIMULTANEOUS EQUATION METHOD)

Formulation	Drug	S. No	Labelled amount (mg/tab)*	Amount found (mg)*	Percentage obtained*	Average	S.D.	% RSD	S.E
OSTIGARD® 100	CEL	1	100	100.41	100.41	100.20	0.298	0.298	0.0083
		2	100	100.28	100.28				
		3	100	100.51	100.51				
		4	100	100.25	100.25				
		5	100	99.66	99.66				
		6	100	100.12	100.12				
	DIA	1	50	49.75	99.51	100.06	0.629	0.628	0.0174
		2	50	49.94	99.88				
		3	50	49.71	99.43				
		4	50	49.95	99.91				
		5	50	50.58	101.17				
		6	50	50.07	100.15				

* Mean of six Observations

TABLE- 4

INTRA DAY AND INTER DAY ANALYSIS OF FORMULATION

(OSTIGARD®) BY SIMULTANEOUS EQUATION METHOD

Drug	Sample No.	Labelled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.		S.E	
			Intra day	Inter day	Intra day	Inter day	Intra day	Inter day	Intra day	Inter day
CEL	1	100	100.35	100.13	0.2847	0.2503	0.2848	0.2505	0.0079	0.0069
	2	100	100.31	100.31						
	3	100	99.78	99.68						
	4	100	99.75	99.75						
	5	100	99.79	99.79						
	6	100	99.80	99.80						
Mean			99.96	99.91						
DIA	1	50	99.64	100.11	0.61838	0.5544	0.6153	0.5510	0.0171	0.0196
	2	50	99.75	99.75						
	3	50	100.88	101.16						
	4	50	100.97	100.97						
	5	50	100.86	100.86						
	6	50	100.84	100.84						
Mean			100.49	100.61						

* Mean of six Observations

TABLE - 5

RUGGEDNESS STUDY (OSTIGARD®)

Drug	**Condition**	**Average*** **% Obtained**	**S.D**	**% R.S.D**	**S.E.**
CEL	Analyst 1 Analyst 2	100.25 100.28	0.0212	0.0212	0.0053
DIA	Analyst 1 Analyst 2	99.88 99.91	0.0212	0.0212	0.005

* Mean of Three Observations

Table - 6

RECOVERY STUDY DATA OF 50 % PREANALYZED FORMULATION

(OSTIGARD®)

Drug	Percentage	Amount present* (mcg/mL)	Amount added (mcg/mL)	Amount estimated* (mcg/mL)	Amount recovered* (mcg/mL)	% Recovery*	S.D	% R.S.D	S.E.
CEL	80	8	6.4	14.4207	6.4207	100.33	0.6638	0.6609	0.0736
	100	8	8	15.9966	7.9954	99.94	0.4951	0.4954	0.01212
	120	8	9.6	17.5663	9.5663	99.64	0.0251	0.0252	0.0027
DIA	80	4	3.2	7.4076	3.2075	100.23	1.4046	1.4013	0.1560
	100	4	4	8.0384	4.0384	100.96	1.1871	1.1755	0.1319
	120	4	4.8	8.8719	4.8729	101.51	0.5322	0.5360	0.05914

* Mean of Three Observations

TABLE-7

OPTICAL PARAMETERS OF CELECOXIB AND DIACEREIN BY AREA UNDER CURVE METHOD

PARAMETERS	CELECOXIB	DIACEREIN
λ_{max}(nm)	236-246	267-273
Beers law limit (µg/mL)	4-20	2-10
Sandell's sensitivity (µg/cm^2/0.001 A.U)	0.002909	0.002515
Molar absorptivity (L mol^{-1} cm^{-1})	134462.3	146866.3
Correlation coefficient (r)	0.9999	0.9999
Regressionequation (y = mx + c)	Y = 0.343758 X + 0.088159	Y = 0.39759X + 0.011842
Slope(m)	0.343758	0.39759
Intercept(c)	0.088159	0.011842
LOD (µg/ mL)	0.057691092	0.003614595
LOQ (µg/ mL)	0.17482149	0.010953318
Standard error of mean of Regression line	0.001008	0.000752

TABLE-8

ASSAY OF COMMERCIAL FORMULATION BY UV- SPECTROSCOPY

(AREA UNDER CURVE METHOD)

Formulation	Drug	S.No	Labelled amount (mg/tab)*	Amount found (mg)*	Percentage obtained*	Average	S.D.	% RSD	S.E
OSTIGARD® 100	CEL	1	100	100.49	100.49	100.48	0.2937	0.2923	0.00815
		2	100	100.99	100.99				
		3	100	100.30	100.30				
		4	100	100.61	100.61				
		5	100	100.18	100.18				
		6	100	100.30	100.30				
	DIA	1	50	50.03	100.07	100.08	0.19935	0.19918	0.005537
		2	50	49.86	99.73				
		3	50	50.10	100.20				
		4	50	49.99	99.99				
		5	50	50.14	100.28				
		6	50	50.10	100.20				

* Mean of six observations

TABLE- 9

INTRA DAY AND INTER DAY ANALYSIS OF FORMULATION BY (OSTIGARD®) AREA UNDER CURVE METHOD

Drug	Sample No.	Labelled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.		S.E	
			Intra day	Inter day	Intra day	Inter day	Intra day	Inter day	Intra day	Inter day
CEL	1 2 3	100 100 100	100.47 100.43 100.52	100.55 100.52 100.55	0.0450	0.0173	0.0448	0.0172	0.0050	0.0019
Mean			100.47	100.54						
DIA	1 2 3	50 50 50	100.09 100.11 100.05	100.03 100.05 100.03	0.0305	0.0115	0.0305	0.0115	0.0033	0.0012
Mean			100.08	100.03						

* Mean of Three Observations

TABLE -10

RUGGEDNESS STUDY BY (OSTIGARD®) AREA UNDER CURVE METHOD

Drug	Condition	Percentage obtained 1	Percentage obtained 2	Percentage obtained 3	Average %	S.D	% R.S.D	S.E.
CEL	Analyst 1	100.65	100.48	100.24	100.45	0.2059	0.2050	0.0228
	Analyst 2	100.05	100.59	100.64	100.42	0.3271	0.3257	0.0363
DIA	Analyst 1	99.96	100.08	100.36	100.09	0.1404	0.1403	0.0156
	Analyst 2	100.01	100.06	99.26	100.01	0.04509	0.04508	0.0050

Table - 11

RECOVERY STUDY DATA OF 50 % PREANALYZED FORMULATION

(OSTIGARD®)

Drug	Percentage	Amount present* (mcg/mL)	Amount added (mcg/mL)	Amount estimated* (mcg/mL)	Amount recovered* (mcg/mL)	% Recovery*	S.D	% R.S.D	S.E.
CEL	80	8	6.4	14.4942	6.4942	101.47	0.3291	0.3257	0.13438
	100	8	8	16.0393	8.0393	100.49	0.1522	0.1512	0.06214
	120	8	9.6	17.7138	9.7138	101.18	0.3291	0.3257	0.13418
DIA	80	4	3.2	7.1977	3.1977	99.92	0.2087	0.2084	0.0852
	100	4	4	8.0196	4.0196	100.44	0.3291	0.3257	0.13437
	120	4	4.8	8.8806	4.8060	100.12	0.2087	0.2084	0.08520

* Mean of Three Observations

TABLE-12

OPTICAL PARAMETERS OF CELECOXIB AND DIACEREIN BY

FIRST ORDER DERIVATIVE METHOD

PARAMETERS	CELECOXIB	DIACEREIN
λ_{max}(nm)	294	245
Beers law limit (µg/ mL)	4-20	2-10
Sandell's sensitivity (µg/cm^2/0.001 A.U)	1.41774	1.0574
Molar absorptivity (L mol^{-1} cm^{-1})	267.915	347.862
Correlation coefficient (r)	0.99995	0.99993
Regressionequation (y = mx + c)	Y = 0.00071X + 0.000029	Y = 0.00095X + 0.000012
Slope(m)	0.00071	0.00095
Intercept(c)	0.000029	0.000012
LOD (µg/ mL)	0.0141385	0.017553624
LOQ (µg/ mL)	0.042843941	0.0531928
Standard error of mean of Regression line	0.000056	0.000045

TABLE-13

ASSAY OF COMMERCIAL FORMULATION BY UV- SPECTROSCOPY

FIRST ORDER DERIVATIVE METHOD

Formulation	Drug	S.No	Labelled amount (mg/tab)*	Amount found (mg)*	Percentage obtained*	Average	S.D.	% RSD	S.E
OSTIGARD® 100	CEL	1	100	99.7468	99.7468	100.33	0.915	0.9120	0.0254
		2	100	101.519	101.519				
		3	100	101.519	101.51				
		4	100	99.7468	99.74				
		5	100	99.7468	99.74				
		6	100	99.7468	99.74				
	DIA	1	50	50.3839	100.76	100.32	1.079	1.075	0.0299
		2	50	50.3839	100.76				
		3	50	50.3839	100.76				
		4	50	49.6622	98.12				
		5	50	50.3839	100.76				
		6	50	50.3839	100.76				

* Mean of six Observations

TABLE- 14

INTRA DAY AND INTER DAY ANALYSIS OF FORMULATION

(OSTIGARD®) FIRST ORDER DERIVATIVE METHOD

Drug	Sample No.	Labelled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.		S.E	
			Intra day	Inter day	Intra day	Inter day	Intra day	Inter day	Intra day	Inter day
CEL	1	100	100.92	100.33	0.2950	0.1732	0.2931	0.1722	0.0081	0.0048
	2	100	100.33	100.33						
	3	100	100.63	100.63						
Mean			100.62	100.43						
DIA	1	50	99.88	99.88	0.2540	0.2540	0.2547	0.2539	0.0070	0.0070
	2	50	99.88	100.32						
	3	50	99.44	99.88						
Mean			99.73	100.02						

* Mean of Three Observations

TABLE -15

RUGGEDNESS STUDY BY (OSTIGARD®) FIRST ORDER DERIVATIVE METHOD

Drug	Condition	Percentage obtained 1	Percentage obtained 2	Percentage obtained 3	Average %	S.D	% R.S.D	S.E.
CEL	Analyst 1	100.70	99.49	99.30	99.83	0.7594	0.7607	0.0843
	Analyst 2	99.71	99.30	100.70	99.90	0.7197	0.7204	0.0799
DIA	Analyst 1	100.41	100.61	99.87	100.29	0.3827	0.3816	0.0156
	Analyst 2	101.07	98.12	100.76	99.98	1.6211	1.6213	0.18012

Table – 16

RECOVERY STUDY DATA OF 50 % PREANALYZED FORMULATION (OSTIGARD®)

Drug	Percentage	Amount present* (mcg/mL)	Amount added (mcg/mL)	Amount estimated* (mcg/mL)	Amount recovered* (mcg/mL)	% Recovery*	S.D	% R.S.D	S.E.
CEL	80	8	6.4	14.5013	6.5013	101.58	0.4365	0.4296	0.04850
	100	8	8	15.919	7.919	98.98	0.3329	0.3363	0.03699
	120	8	9.6	17.4785	9.4785	98.73	0.1527	0.1547	0.01697
DIA	80	4	3.2	7.2029	3.2029	100.09	0.9200	0.9191	0.10222
	100	4	4	8.0488	4.0488	101.22	0.5900	0.5828	0.06550
	120	4	4.8	8.8947	4.8347	100.72	0.2730	0.2710	0.03033

* Mean of Three Observations

TABLE - 17

SYSTEM SUITABILITY PARAMETERS FOR THE OPTIMIZED CHROMATOGRAM BY RP - HPLC

PARAMETERS	CELECOXIB	DIACEREIN
Tailing factor	1.50	1.22
Asymmetrical factor	1.58	1.26
Theoretical plates	2965	1506
Theoretical plate per unit Length	197.66	100.40
Resolution	Between DIA and CEL 7.15	

TABLE - 18

OPTICAL CHARACTERISTICS OF CELECOXIB AND DIACEREIN BY RP - HPLC

PARAMETERS	CELECOXIB	DIACEREIN
λ_{max} (nm)	252	252
Beers law limit (µg/ mL)	70 -130	35 – 65
Correlation coefficient (r)	0.9998	0.9996
Regression equation (y=mx+c)	y= 16649.2393x + 272062.6429	y=33258.0643 x + 68821.3571
Slope (m)	272063	-68821
Intercept (c)	16649	33258
LOD (µg/ mL)	0.00025	0.0001606
LOQ (µg/ mL)	0.00078	0.0004866
Standard Error	0.15368	0.1143154

TABLE – 19

QUANTIFICATION OF FORMULATION (OSTIGARD®) BY RP – HPLC

Drug	Conc.	Sample No.	Labelled amount (mg/tab)	Amount found (mg)	Percentage obtained	Average	± S.D	% R.S.D	S.E.
DIA	Low level (80%)	1	100	98.92	98.92	99.14	0.1777	0.1795	0.0197
		2	100	98.84	98.84				
		3	100	99.18	99.18				
	Mid level (100%)	1	100	98.84	98.84	98.84	0.1600	0.1618	0.0177
		2	100	99.00	99.00				
		3	100	98.68	98.68				
	High level (120%)	1	100	99.00	99.00	98.95	0.0404	0.0408	0.0044
		2	100	98.92	98.92				
		3	100	98.95	98.95				
ACE	Low level (80%)	1	50	49.90	99.80	99.83	0.0550	0.0551	0.0061
		2	50	49.89	99.79				
		3	50	49.95	99.89				
	Mid level (100%)	1	50	49.81	99.62	99.70	0.0529	0.0531	0.0058
		2	50	49.97	99.94				
		3	50	49.77	99.54				
	High level (120%)	1	50	49.80	99.60	99.76	0.1539	0.1543	0.0171
		2	50	49.95	99.90				
		3	50	49.90	99.81				

TABLE – 20

RECOVERY ANALYSIS OF FORMULATION (OSTIGARD®) BY RP - HPLC

Drug	Percentage	Amount present* (mcg/mL)	Amount added (mcg/mL)	Amount estimated* (mcg/mL)	Amount recovered* (mcg/mL)	% Recovery*	S.D	% R.S.D	S.E.
CEL	+10 spiked	100	10	108.92	9.947	99.47	0.2214	0.2222	0.02460
	+20 spiked	100	20	118.95	19.976	99.88			
	+30 spiked	100	30	128.84	29.858	99.53			
DIA	+10 spike	50	5	54.86	4.980	99.61	0.2569	0.2575	0.02855
	+20 spike	50	10	59.87	9.992	99.62			
	+30 spike	50	15	64.89	15.009	100.06			

* Mean of Three Observations

BIBLIOGRAPHY

7. BIBLIOGRAPHY

1. Anonymous.http://icn2.umeche.maine.edu/newnav/NewNavigator/images/UV-visible_intro%202.htm

2. Anonymous.http://teaching.shu.ac.uk/hwb/chemistry/tutorials/molspec/uvvisab1.Html

3. Anonymous. http://teaching.shu.ac.uk/hwb/chemistry/tutorials/molspe/uvvisa1.htm

4. Anonymous. Instruction Manual. Shimadzu Solvent Delivery Module LC-10ATvp. Shimadzu Corporation, Kyoto, Japan, **2001**, 8.3 - 8.38.

5. Anonymous. Remington. The Science and Practice of Pharmacy. 21st edn., Wolters Kluwer Health (India) Pvt. Ltd., New Delhi, **2007,** (I), 633-642.

6. Anonymous. Shimadzu Instruction manual pharamaspec UV-1700 series operation guide, Shimadzu Corporation, Kyoto, Japan, **2001,** 6.2.

7.Anonymous. The Merck Index. 14th edn., Merck Research Laboratories Division of Merck & Co. Inc, USA, **1958**, 2961.

8.Anonymous. www.chem.utoronto.ca/edu/ac.

9.Anonymous. www.chemistry.bd.psu.edu/justik.

10. Anonymous. www.chromotech.com

11. Anonymous. www.drugbank.ca/drugs.

12. Anonymous.http://icn2.umeche.maine.edu/newnav/NewNavigator/images/UV-visible_intro%202.htm

13. Anonymous. Indian Pharmacopeia vol 2. The Indian Pharmacopeia Commission Ghazidabad, **2010**, 1191 – 1194.

14. Anonymous. www.druglib.com/druginfo/pleat/warings, precautions/22k
15. Archana Roy, Daisy Yohannan, Lalith K and Ranendra N. Saha. Development of Rapid Spectrophotometric Methods for Estimation of Celecoxib and Acyclovir in formulations, *Indian Journal Pharmaceutical Education Research*, **2008,** 42(3) 215-221.
16. Beckett A. H and Stenlake J. B. Practical Pharmaceutical Chemistry. 4th edn., CBS Publishers and Distributors, New Delhi, **2007**, (II), 259-260, 278-290, 293-297.
17. Chandran S, Jadhav PR, kharwade PB and Saha RN. "Rapid and sensitive spectrofluorimetric method for the estimation of celecoxib". *Indian Journal of Pharmaceutical Sciences*, **2006**, 68(1), 20-25.
18. Chatwal G and Anand. Instrumental Methods of Chemical Analysis. 5th edn., Himalayan Publishing House, Mumbai, **2007**, 1.2, 2.108, 2.168, 2.147.
19. Code Q2A, Text on Validation of Analytical Procedures. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, **1994**, 1 - 5.
20. Code Q2B, Validation of Analytical Procedures; Methodology. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, **1996**, 1 - 8.
21. Deshpande M. M., Gondane S. J, Mahajan M.P., Deshpande A. S and Sawant S.D. Estimation of Diacerein by UV Spectrophotometry in Bulk and formulation. *International Journal of Chemical and Analytical Science*, **2011**, 2(1), 1-2.
22. Douglas A. Skoog, Donald M. West, James E. Holler and Stanley Crouch. Fundamental of Analytical Chemistry. 8th edn., Thomson Asia Pvt. Ltd., Singapore, **2006**, 2, 344-346, 677.

23. Giannellini V, Salvatore F, Bartolucci G, Coran SA, Bambagiotti-Alberti M. A validated HPLC stability indicating method for the determination oof diacerhin in bulk drug substance *J Pharm Biomed Anal.* **2005**, 39 (3-4), 776-780.

24. Jadhav A. S and Shingare M. S. A New Stability Indicating RP-HPLC Method to Determine Assay and Known Impurity of Celecoxib API. *Drug Development and Industrial Pharmacy*, **2005**, 31 (8), 779-783.

25. Janhavi rao, Kanchan Chauhan, Mahadik K.R and Kadam S.S. "Stability-Indicating high performance liquid chromatographic method for the determination of Diacerein in capsules"**.** *Indian journal of pharmaceutical science*. **2009,** 24-26.

26. Jayasagar G, Kumar MK, Chandrasekhar K, Prasad PS, Rao YM. Validated HPLC method for the determination of celecoxib in human serum and its application in a clinical pharmacokinetic study. *Pharmazie.* **2002**, 57(9), 619-21.

27. Kannappan N, Madhukar A, Srinivasan R, Srinivas R.L.A, Naveen Kumar CH and Mannavalan R. Analytical Method Development and Validation of Diacerein tablets by RP-HPLC. *International Journal of ChemTech Research,* **2010**, 2(1), 143-148.

28. Lalitha Keddal G, Thangavel Venkatachalam, Ramraj Srinivasan, ponnusamy Kalaiselvi, Nagappan Kannappan. A Simple HPLC Method for Quantiation of Diacerin in Tablet Dosag Form. *European Journal of Analytical Chemsty,* **2010,** 5(1), 81-88.

29. Lloyd, R. Sydner, Joseph, J. Kirkland, and Joseph, L. Glajch. Pratical HPLC Method Development. 2nd edn., John Wiley and Sons, Inc., USA, **1997**, 22-24, 42, 235-240.

30. Murthy T.E.G.K, chowdary Y.A, Narendra Kumar K, Gowri Sankar D, and Durvasa Rao B. "Reverse Phasse HPLC Determination of Celecoxib in Dosage Forms". *The Pharma Review,* **2006,** 97.

31. Neelam Seedher, Garg A, and Sinu Bhatia,"Spectrophotometric Method for Estimation of Some COX-2 Inhibitors in pure and Pharmaceutical Formulations". *Indian Journal Pharmaceutical Sciences***, 2003**, 12-16.

32. Sarika Narade, Snehal Patil, Sharda Surve, Dhanashri Shete and Yogesh Pore. Development and Validation of UV Spectrophotometric Method for the Determination of Diacerein in Capsules. *Digest Journal of Nanomaterials and Biostructures*, **2010**, 5 (1), 113 – 118.

33. Sekar V, Jayaseelan S, Udhaya Kumar E, Subash N, Prakash M and Perumal P. Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Diacerein and Aceclofenac in Dosage form. *International Journal of ChemTech Research*, **2010,** 2 (1), 168-171.

34. Sethi P. D. Quantitative Analysis of Drug in Pharmaceutical Formulations, 1st edn., CBS Publishers and Distributors, New Delhi, **2001**, 7-13.

35. Sivakumar R., Nallasivan PK., Saranya KC., Solomon WD Sam., Akelesh T and Venkatnarayanan R. Visible Spectrophotometric Estimation of Diacerein in Bulk and Pharmaceutical Dosage Forms. *Journal of Young Pharmacists*. **2010**, 2(4), 414–416.

36. Sreejith K.R and Dr. Premalatha K. Novel Spectrophotometric Methods for Estimation of Diacerin from Formulations. *International Journal of Research in Pharmaceutical and Biomedical Science*, **2011**, 2(3), 992-999.

37. Sunil kumar dubey, Chiranjeevi Venkatesh K and Jangala Hemanth. Spectrofluorimetric determination of Celecoxib in bulk and formulations. *Americian Journal of Pharmtech Research*, **2011** volume 1 (3), 128 – 137.