Development and Pharmacological Evaluation of Sustain Release Based Formulation of a Non Steroidal Anti-Inflammatory drugs

Thesis submitted by

Soumen Mukhopadhyay

Doctor of Philosophy (Pharmacy)

Department of Pharmaceutical Technology

Faculty of Engineering & Technology

Jadavpur University

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Soumen Mukhopadhyay, M. Pharm (JU)
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Jadavpur University
Kolkata -700032.

Name, Designation & Institution of the Supervisor :

Prof.(Dr.) Tapan Kumar Chatterjee
Former Professor,
Dept.of Pharmaceutical Technology,
Jadavpur University
Kolkata

List of Publication :- (see annexure for details)

i) **"PREPARATION AND EVALUATION OF DIACEREIN LOADED MICROSPHERE WITH SODIUM ALGINATE AND ETHYL CELLULOSE BY DOUBLE EMULSION SOLVENT EVAPORATION METHOD"**

Soumen Mukhopadhyay, Sharmily Chakraborty **,**Sumuntha Das and Dr. Tapan Kumar Chatterjee, **Int J Pharm Bio. Sci. 2016; 7 (3): 231-239**

ii) **"FORMULATION DEVELOPMENT AND EVALUATION OF DIACEREIN LOADED MICROSPHERE BY SPRAY COATING (WURSTER METHOD)"**

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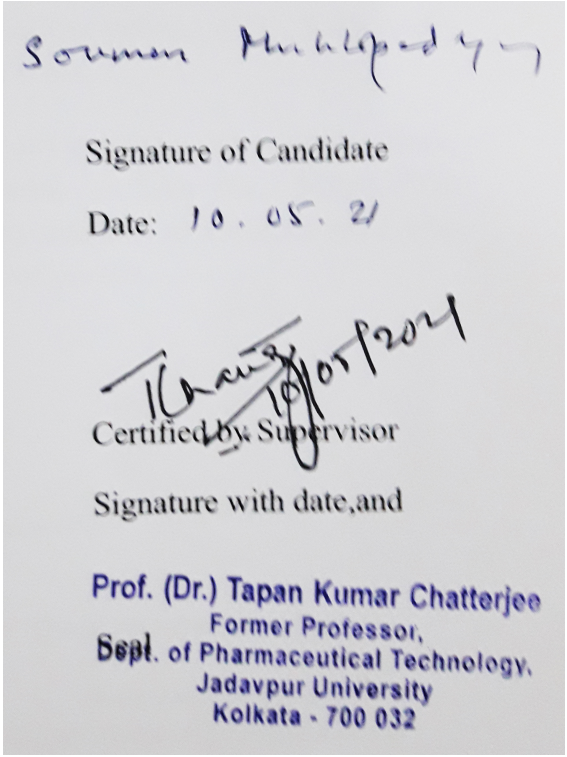
Declaration of originality and compliance of academic ethics :

I **Soumen Mukhopadhyay** registered on August 26, 2015, Ref No. D-7/E/516/15 dt 21.08.2015, do hereby declare that this thesis entitled "**Development and Pharmacological Evaluation of Sustain Release Based Formulation of a Non Steroidal Anti Inflammatory drugs"** contains original research work done by the undersigned as part of Doctoral Studies.

In accordance with existing academic rules and ethical conduct, all information in this thesis have been presented. This is also to state that I had clearly mentioned all references, experimental findings, results that are not original in this research work. I also declare that I checked this thesis as per the "Policy on Anti Plagiarism, Jadavpur University, 2019", and the level of Similarity as checked by; iThenticate Software is 05%-.



Name : Soumen Mukhopadhyay

Registration No ;Ref No. D-7/E/516/15
Dt. 21.08.2015

Title of the thesis : Development and Pharmacological Evaluation of Sustain Release Based Formulation of a Non Steroidal Anti-Inflammatory drugs

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Date

10.05.21

Soumen Mukhopadhyay

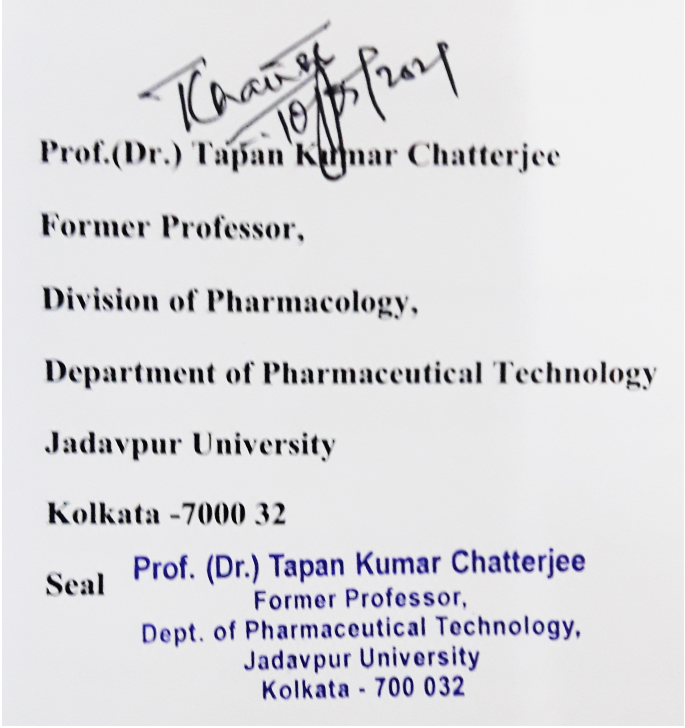
(Soumen Mukhopadhyay)

Research Scholar
Department of pharmaceutical Technology
Jadavpur University
Kolkata-700032
India

CERTIFICATE FROM THE SUPERVISOR

This is to certify that the thesis entitled "**Development and Pharmacological Evaluation of Sustain Release Based Formulation of a Non Steroidal Anti - Inflammatory drugs**" submitted by Shri Soumen Mukhopadhyay who got his name registered on 26th August 2015, Ref No. D-7/E/516/15 dated 21.08.2015, for the award of PhD.(Pharmacy) degree of Jadavpur University is absolutely based upon his own work under the supervision of the undersigned and that neither his thesis nor any part of the thesis has been submitted for any degree/diploma or any other academic award anywhere before.

Signature of the Supervisor and Date with office seal



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ABSTRACT

Diacerein is a slow acting drug having anti inflammatory anti osteoarthritic action. It is a drug under the class of anthraquinone derivative; It is believed to act via inhibition of 1-L-1 Beta, a protein responsible for pain, inflammation and cartilage destruction in osteoarthritis. Drug action is relatively short, almost insoluble in aqueous medium. Via oral route the drug is poorly bioavailable (approx 35 to 56 %).

Diacerein microsphere was prepared by two different methods e.g. spray coating (wurster method) and double emulsion solvent evaporation method with an objective to manufacture sustained release formulation. Assay of microsphere was done by HPLC method and drug loading and drug entrapment efficiency were also calculated. Surface topography of the microspheres were determined by Scanning Electron Microscopy (SEM study).

Particle size, Size distribution, Zeta potential and poly disparity index of Diacerein microspheres were measured by Dynamic Light Scattering technique.

FTIR study, X-Ray diffraction and Differential Scanning Calorimetry (DSC study) of the microspheres were done to establish drug polymer and other excipients compatibility and stability.

In-vitro dissolution rate study of Diacerein microsphere was done in phosphate buffer (pH6.8) and citrate buffer (pH 6.0) and 0.1N Hcl to establish its sustained release action and compared with drug release from conventional Diacerein capsule available in the market.

Release kinetics were also studied by different mathematical model like zero order, first order, Higuchi, Korsmeyer-Peppas and Hickson- Crowell model. Study of regression-coefficient of different models showed best fit model for release of drug from microsphere

Pharmacokinetic parameters like C_{max}, T_{max}, AUC $_{0\text{-}t}$, AUC $_{0\text{-}\infty}$,T $_{½}$, k_{el} of the Diacerein microspheres were determined in rabbit plasma and compared with marketed capsule formulation. This in-vivo study also showed sustained release drug delivery from the microsphere.

Good in-vitro and in-vivo correlation was seen.

SYNOPSIS

The present thesis entitled “Development and Pharmacological Evaluation of Sustain Release Based Formulation of a Non Steroidal Anti-Inflammatory drugs”, deals with the formulation development of Diacerein microsphere by Spray Coating (wurster method) and also by double emulsion solvent evaporation method. Diacerein, an anti inflammatory, anti osteoarthritis drug was chosen for microsphere formulation. Hydroxy propyl methyl cellulose 6cps (|HPMC) and ethyl cellulose N7 (EC) were used as coating polymer in spray coating method and Sodium alginate and ethyl cellulose were used as coating polymer for double emulsion method.

Chapter1: Introduction, Microsphere as drug delivery system is shown in this chapter where development and characterization of microsphere and its importance and use as sustained release drug delivery is described from different review articles published from time to time.

Chapter 2: shows importance of sustained release and controlled release drug delivery system from different published papers and review articles.

Chapter 3: Describes the nature of the disease Osteoarthritis, its signs and symptoms, Risk factors, Patho physiology. Diagnosis, , Management, Medication and Research were shown from different published papers and research articles.

Chapter 4: Describes Physiochemical parameters of Diacerein its pharmacology, Distribution Metabolism, Dose, Side effects, Special precaution, pharmacokinetic and pharmacodynamic parameters are shown .

Chapter 5: Shows a brief literature survey done to enumerate different published work on microsphere as a drug delivery system.

Chapter 6 : List of tables of different experiments, Materials, Method, Instruments and equipment are described in this chapter

Manufacture of Diacerein microsphere and its evaluation was also mentioned

Chapter 7

Shape and morphology of Diacerein microsphere was shown by SEM study,

Chapter-8

Measurement of Particle size of Diacerein microsphere, Size distribution, Zeta potential, poly dispersity index were described.

Chapter-9

Stability and compatibility of drug , polymer and excipients were studied by FTIR, XRD,&DSC study.

Chapter-10

In-vitro drug release study of Diacerein microsphere in different buffered medium and comparison with conventional Diacerein capsule available in the market were studied and also study of release kinetics from the microsphere were done.

Chapter-11

In - vivo Pharmacokinetic study of Diacerein microsphere in rabbit plasma and comparison with conventional Diacerein capsule was shown.

Chapter-12

Results of all investigations undertaken were presented in an orderly manner under Results and Discussion. The findings were discussed with sound theoretical basis and enough evidence. Based on the entire research work conclusion was drawn.

Chapter 13 Enlists all the references cited in the text of this thesis book.

Annexure:-

First page of published papers (1&2) & 3. Abstract submitted in a seminar

Why Diacerein was chosen as a model drug for microsphere preparation ?

(i) Diacerein is a relatively short acting drug with Terminal elimination half life 4.3 hour. Oral bio availability of Diacerein is poor 35 to 56% though standard meal increases bio availability but delays systemic absorption. Generally prescribed as 50 mg twice daily

(ii) Diacerein is practically insoluble in water. (approx 3.1 mg/L at 20^{0C}).

(iii) Maximum plasma concentration of Diacerein (C_{max}) after 50 mg human dose is 3.2 mg/Litre

(iv) Time to reach maximum plasma concentration (C_{max}) in human is 2.2 hour(T_{max}). This is increased by food.

(v) Conventional Diacerein capsule (50mg) releases it drug content, up to 80% in one hour (In-vitro drug release in phosphate buffer 6.8). Major side effect of Diacerein is diarrhea or soft stood.

(vi) Diacerein loaded microsphere having sustained release action was prepared with the objective to release drug over a longer period of time and prevents dose dumping, better pain management, reduce side effects.

(vii) Diacerein loaded microsphere with sustained release action will have lower elimination rate and longer half life.

Aims & Objective of the study:

The main objectives of the study can be highlighted as follows:-

i) To formulate Diacerein microsphere with sustained release action by different method.

ii) To study the surface topography of the prepared Diacerein microsphere.

iii) To study the particle size, size distribution zeta potential and poly dispersity index (PDI) of microsphere.

iv) To study the X-ray diffraction pattern (powder X-ray diffratometry), Infra-red (FTIR) Spectrum and differential scanning Calorimetry (DSC) of pure drug, Diacerein microsphere and drug excipients physical mixture to analyze whether any complex formation occurred between the drug and the polymer or between the drug and the excipients.

v) In-vitro dissolution rate study of Diacerein microsphere in different medium like phosphate buffer, citrate buffer acidic medium to study sustained release action of the formulation. And comparison with conventional Diacerein capsule available in the market.

vi) Compilation of drug release data in the light of different kinetic models like (a) Zero order, (b) First order, (c) Higuchi, (d) Koresmeyer & peppas (e) Hickson-crowell model and identification of best fit model.

vii) To study pharmacokinetic pattern of Diacerein microsphere in rabbit plasma and comparison with marketed Diacerein capsule formulation.

viii) To establish a relationship between in-vitro drug release and in-vivo absorption of Diacerein microsphere.

LIST OF ABBREVIATIONS

ANOVA	**–**	**Analysis of Variance**
API	**-**	**Active Pharmaceutical Ingredient**
AUC	**-**	**Area Under Curve**
ADAMPT	**-**	**A disintegrin and metelloproteinage with thrombspondin motits.**
DCN	**-**	**Diacerein**
DSC	**-**	**Differential Scanning Calorimetry**
EC	**-**	**Ethyl Cellulose**
FTIR	**-**	**Fourier Transform Infrared Spectroscopy**
HPMC	**-**	**Hydroxy Propyl Methyl Cellulose**
HQC	**-**	**High Quality Control Sample**
HPLC	**-**	**High Performance Liquid Chromatography**
I-L-1ß	**-**	**Interleukin – 1 Beta**
IS	**-**	**Internal Standard**
LQC	**-**	**Low Quality Control Sample**
LOQ	**-**	**Lower limit of Qualification**
MQC	**-**	**Medium Quality Control Sample**
MMP	**-**	**Matrix Metalloproteinase**
MCC	**-**	**Micro Crystalline Cellulose**
OA	**-**	**Osteoarthritis**
RA	**-**	**Rheumatoid Arthritis**
SEM	**-**	**Scanning Electron Microscopy**
SR/CR	**-**	**Sustained Release/ Controlled Release**
UV	**-**	**Ultraviolet Spectroscopy**
XRD	**-**	**X- Ray diffraction**

CHAPTER-1

INTRODUCTION

Literature survey on microsphere as drug delivery system (1):-

Definition of microspheres states that it is a rigid nearly spherical particles having size from 1 to 1000 µm (1mm). which means microspheres are spherical particles of micrometer size range. In case of injectable preparation microspheres sometimes have size less than 200µm.

The role of microsphere as particulate drug delivery system is very importane. Which are enumerated in this chapter.

The microspheres are similar to microcapsule. Generally microspheres are solid or porous in nature and do not have fluid inside, as opposed to microcapsules which are hollow and may contain fluid inside.

Microsphere and Microcapsule are the types of Microparticle which may be manufactured following different methods. However, their final properties depend on their composition and elaboration procedures..

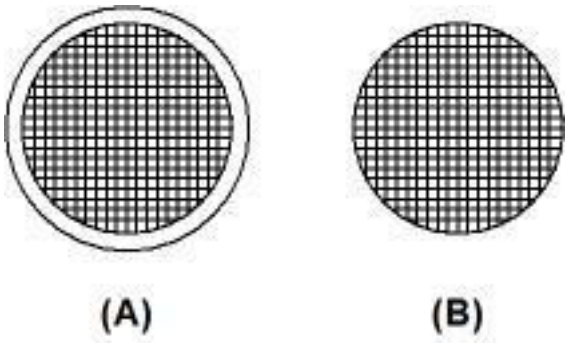


Figure 1.1: Diagrammatic illustration of Microcapsule (A) containing a microsphere having difference between the core and coating zone.(B) Matrix microcapsule or microsphere.

Microsphere have various commercial applications, such as Polystyrene microspheres are used in biomedical applications for e.g. Cell sorting, immune preparation and medical research. Glass microspheres are used as filler for weight reduction, additive for cosmetic.

Ceramic microspheres are used as grinding media, Polythene microspheres are used as permanent or temporary fillers

Advantages and disadvantages of microspheres over conventional dosage forms: (4)

There are plenty of advantages of microsphere over other formulation. It can provide extended and constant release of drugs and therefore used in SR/CR formulations and asa result of which therapeutic effect is extended. It can lower dosing frequency and increase patient compliance. Drug absorption from microsphere is more reproducible. With microsphere better therapeutic effect is achieved in case of short half life drugs Microsphere reduces dose dumping. It can mask bad taste and odour.First pass metabolism of drugs is avoided in microsphere. It can be used in injectable formulation due to its small and spherical size. Microsphere can improve biological half- life and can improve bioavailability.

Microsphere formulation also has some disadvantages. In case of any loss in integrity of drug release pattern, potential toxicity may be caused. From one to another dosage form release pattern may be different. Microsphere as dosage form can not be crused or chewed.

Preparation methods of microsphere:

I) Spray drying
II) Single emulsion
III) Double emulsion solvent evaporation
IV) Phase separation coacervation
V) Polymerization
VI) Spray coating (wurster method)

Application of microsphere in pharmaceutical industry for delivery of drugs :

Oral, Ophthalmic, Buccal, Nasal, Intratumoral, gastrointestinal, Transdermal, Colonic, Vaginal, Local drug delivery etc.

Microspheres are of various types such as

a) Bioadhesive. b) Magnetic. c) Floating. d) Radioactive. e) Polymeric. f) Biodegradable.

Different types of polymers used for preparation of microsphere.

a) Natural b) Synthetic

Natural polymers like Carbohydrates, protein, Chemically modified Carbohydrates.

Agarose, Carrageenan, Chitosan, Starch are the examples of Carbohydrate.

Albumin, collagen, Gelatin are the examples of protein. Poly dextran, poly starch are chemically modified carbohydrates.

Moreover synthetic polymers e.g. biodegradable polymers and non biodegradable polymers are also used in microsphere formulations.

Acrolin, Poly(methyl methacrylate) (PMMA), Epoxy polymers are examples of non biodegradable polymers.

Different types of drug release pattern from microsphere.

Drug release mechanism from microsphere are dissolution, dliffusion, Osmotically, driven release & erosion. During the drug release phase above mechanism occurs parallelly. One or other mechanism play a greater role. Osmetic pressure has to be taken into account for semi permeable coating. Dissolution profile may be accelerated in case of use of water soluble pore formers by creating pores. Drug release takes place via a stimulus in case of a smart drug delivery microparticle. Number of stimulus required for dissolution may be one, two or multiple stimulus for drug release can be internal or external classified as physical, chemical or microbiological.

In case of dissolution mechanism, drug or API is encapsulated by a matrix of polymer and properties of polymer is pivotal at the time of dissolution. Drug properties be dependent on dissolution medium and formulation. In event of diffusion, API can be passed through the intact polymer network or water filled pores. Drugs which are aqueous soluble may dissolve in aqueous pore net works. Due to water uptake polymer chain swells up indicating formation of new pores and /or osmotic pressure.

Increase in volume due to swelling may cause increase in diffusion co-efficient of drug.

Entrapped API

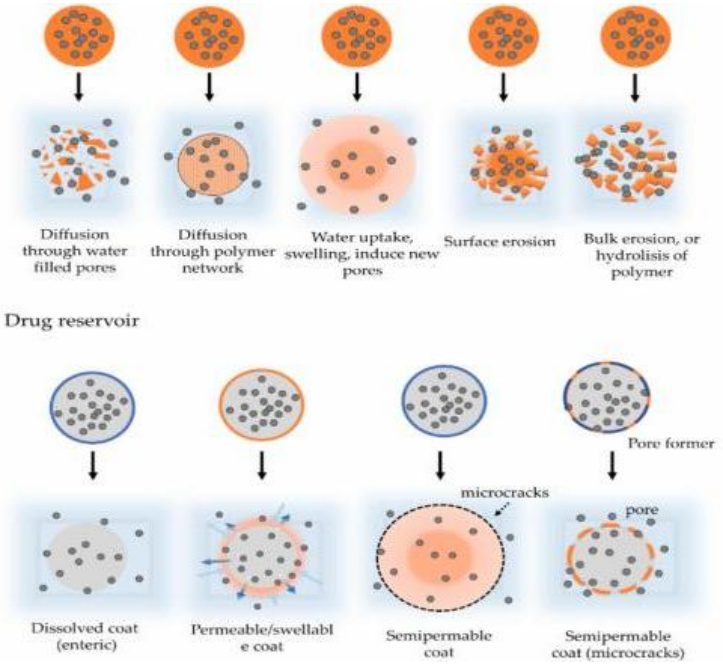


Figure 1.2 : Release mechanisms in microencapsulated products.

Stimuli responsive polymer

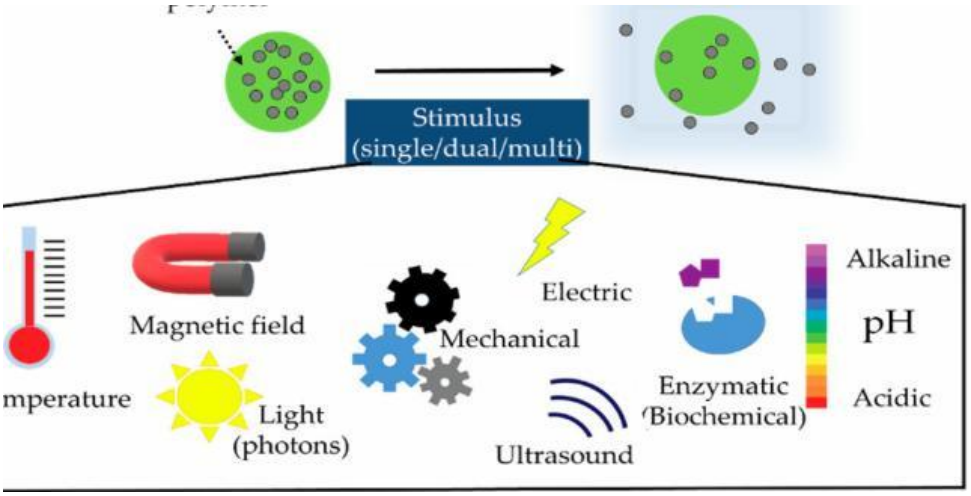


Figure 1.3: Release of drug from microcapsule via stimuli

Physiochemical evaluation of micro particulates :-

i) Determination of size of the particle, distribution, shape of particle of micro particulates can be measured by optical microscope with calibrated eyepiece micrometer, measurement of 100 microspheres average particle size can be determined. Dynamic Light scattering technique is a method which determines particle size, size distribution, Zeta potential and polydispersity index..

ii) Surface topography of microsphere scanning electron microscopy is used to determine shape and morphology of microparticulates. Microsphere surfaces can be investigated by SEM method and can also be used for double walled systems.

iii) Determination of density: Density of microspheres is determined by multi volume pycnometer. Densitometer can also be for this purpose.

iv) Porosity measurement by porosimetry: Water uptake, swelling, reconstitution followed by drug release in thus, porosity of microparticles has significant role. Porosity of microparticles can be measured by Murcury porosimeter.

v) Measurement of Iso electric point: Electrophorectic Mobility of microspheres can be measured by using microelectrophoresis apparatus. By measuring the particle movement over a distance of 1 mm, mean velocity at different pH value from 3-10 is calculated. (4)

vi) Angle of contact: Wetting property of microparticulate system is determined with the help of angle of contact whether the microsphere is hydrophilic or hydrophobic can be understood. (4).

vii) Electron Spectroscopy: Electron spectroscopy is used to determine the surface chemistry of the microsphere. It helps to provide a mean to determine atomic composition of the surface. For biodegradable microsphere it can help to know surface degradation (2)

viii) DSC thermogram: Differential Scanning Calorimetry(DSC) can be useful for analyzing drug-polymer interaction, in the characterization of microsphere. DSC thermogram of API, microsphere, drug polymer and other ingredients physical mixture can be studied for characterization.

ix) Spectrometry: FTIR and powder X-ray diffratometry (PXRD) analysis performed in microsphere showed intra molecular changes, drug polymer interaction and degradation in microspheres (9).

x) Assay: Drug content in microsphere can be assayed by HPLC method and/or UV Spectrophotometry.

xi) Drug entrapment efficiency: (9)

EE% = Entrapped drug content/theoretical drug content x 100

xii) Drug release study (2): Dissolution rate in- vitro of drug from microsphere is a very useful procedure for control of quality in drug manufacture and development of formulation. USP and BP dissolution apparatus are used to study in- vitro release of drug by the use of rotating elements Paddle and Basket and dissolution medium like different buffers, phosphate, citrate, acetate etc are used for the release study.

xiii) In- vivo study :-This procedure involves anesthetizing the animal followed by administration of dosage form through oral route and withdrawing blood at different time intervals. Animal models like rabbit, rat, mice, dog, sheep etc are used. Plasma drug content is analyzed using instruments like HPLC, LCMS etc. (4).

CHAPTER-2

LITERATURE SURVEY ON SUSTAINED / CONTROLLED RELEASE FORMULATION

In current years with the advancement in formulation development, there has been a growing trend to manufacture sustained/controlled release formulations to different existing and new drugs. Consequently, more and more sustained/controlled release new formulations are getting introduced in the market. The popularity of prolonged release dosage forms is due to its safety and efficacy over immediate release drugs e.g reduction of frequency of dosing, increase in efficacy, reduction in adverse effects (3).

Modern research in health care system also has been extensively directed towards a better understanding of the mechanism of drug absorption from various routes of drug administration. Parallel research has explored some of the negative aspects of drugs at absorption sites and approaches to minimize such injury. Such knowledge has led in a great many cases to a more rational design of prolonged action dosage forms. Hence, although new drug molecule and their derivative still receive priority from most drug manufacturers, there is increasing interest in development of new drug delivery, such as sustained/ controlled release dosage forms.

Another factor, which develops interest in prolonged release dosage forms is rapid growth of polymer technology and its application to the solution of some biomedical problems.

The conventional drug delivery system produces wide fluctuation in plasma drug concentration, requires patient to remember the dosage schedule, may miss a particulate dose. Attainment of steady state plasma concentration is difficult due to fluctuation of plasma drug concentrations. Sometime produces undesirable adverse action and poor therapeutic effect(5). Maintenance of desired plasma drug concentration is most important for proper treatment. Factors like repetitive drug administration to the patient and unforeseeable drug absorption lead to relatively newer concept of oral sustained or controlled release drug delivery system. (1) Fluctuation in drug concentration level in conventional dosage form is indicated in figure 2.1

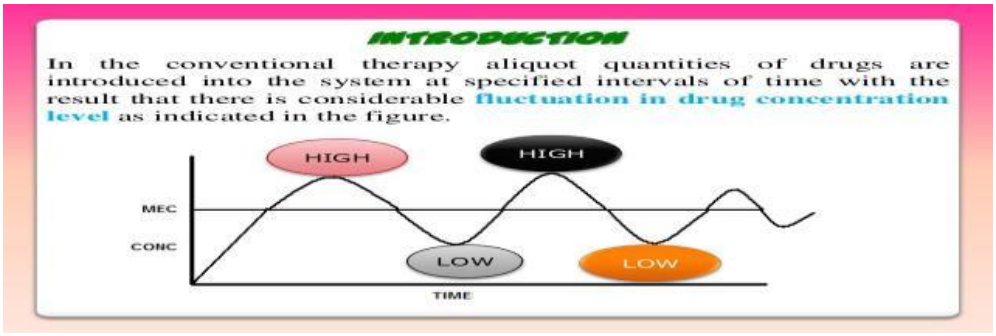
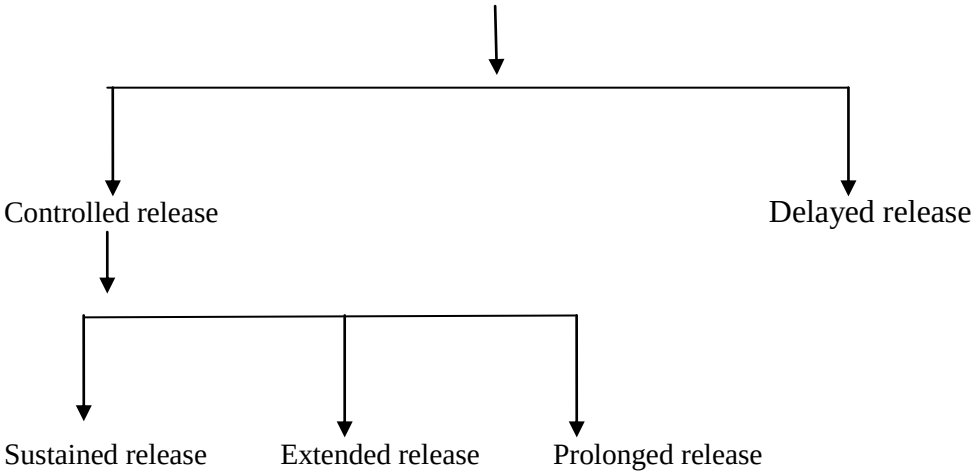


Figure: 2.1

Ideal dosage form can carry the active drug or prodrug at a rate indicated as per requirement of the system over the span of treatment. It should carry the drug at the place of action. This can be attended by the development of modified release dosage form. Modified release dosage form can be subdivided as under;



For the last few decades conventional drug delivery systems are largely being replaced by new and novel dosage forms and most common among them are controlled/sustained release dosage form and which have become very popular in modern day treatment. (4).

By definition sustained release or controlled release drug exarts therapeutic effect for long time by set free the drug for prolong time period when the drug is administered as single dose to the patient. Many abbreviations like ER, SR, XL etc. are used to denote this type of drug delivery system(2).

On other hand definition of controlled release drug states that the science by which therapeutically effective constitutients of drug could be available to the site of action gradually and can exert the desired therapeutic action.

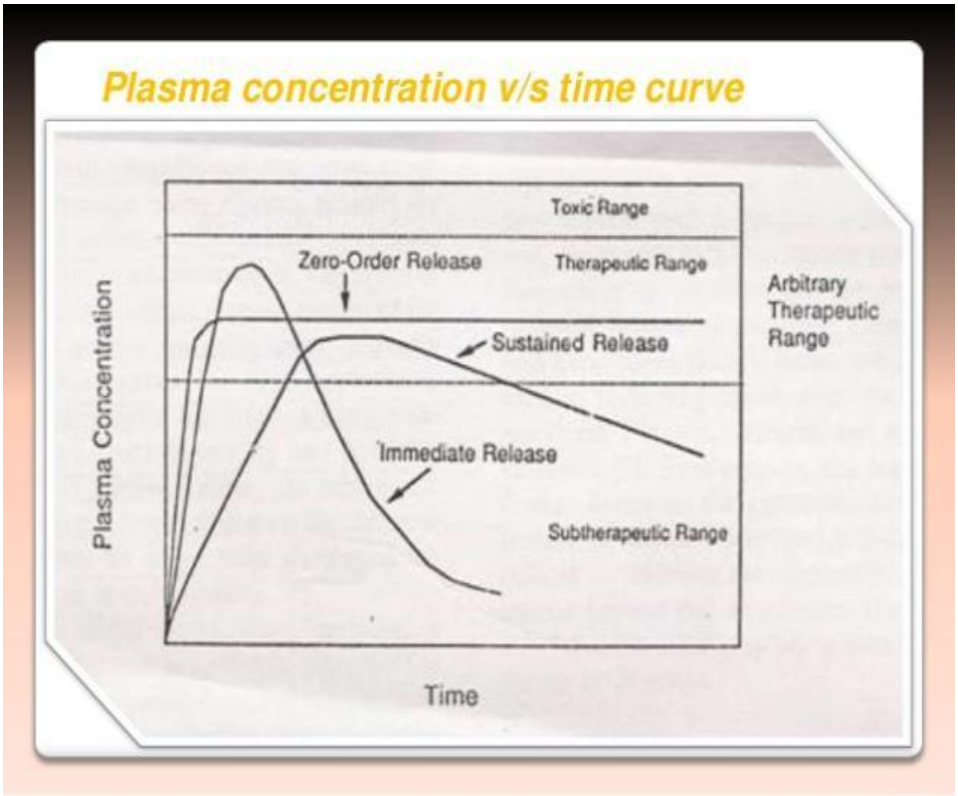


Figure 2.2

Comparative plasma concentration of instant release, sustained release and controlled release drug.

Advantages & disadvantages of sustained (SR)/ Controlled (CR) release dosage form:-

The Advantages of SR/CR dosage forms are maintenance of constant therapeutic level. Reduction of dosage frequency. Pharmacological response become uniform. Total drug used may be reduced. Adverse effects may be reduced. Drug action may be obtained at the site of action. Fluctuation of plasma concentration may be reduced. Patient compliance may be increased. More uniform compared to conventional dosage form (6).

Disadvantages are reduced potential for dosage adjustment, increased chances of first pass clearance, environmental factors can cause the premature and exaggerated release of a drug. It can increase cost of formulation. Sometime poor in-vitro invivo co-relation (4).

Sustained release drug delivery system can be classified as Diffusion, Dissolution, Ion exchange and Osmotic pressure.

Basis on which a drug to be met to formulate SR/CR dosage form (6).

i) Drug should have high therapeutic index
ii) Drug should have advantageous half life
iii) Dose should be small
iv) Should have advantageous absorption and solubility characteristics.
v) Absorption window should be prudent
vi) Drug should have first pass Clarence.

To design a CR dosage form what factors can influence. The factors which can influence are physiological properties for e.g. Aqueous solubility and biological factors. Partition co-efficient (P-Value), Drugs having high or low P-Value are not suitable for CR dosage form. P-Value means fraction of a drug soluble in Oil phase or aqueous phase. It is an important factor which affects passive diffusion of drug across the biological membrane. Drugs having solubility in both phase are desirable. Another factor is Pka value of a drug which determines ionization at physiologic pH in GIT. Pka of a drug defines the point in which it is 50% ionized. If the drug is highly ionized it is not a suitable candidate for CR dosage form. Through a biological membrane absorption of unionized drug occurs rapidly compared to ionized drug (6).

Drug stability is one of the important factor for the design of CR dosage form. Acid base and enzymatic degradation stable drugs are suitable candidate for CR dosage from. Drugs which degrades in stomach or small intestine are not suitable for CR formulation as it lower the bioavailability of the concerned drug.

Molecular size and molecular weight are other two important factor for CR formulation. Drugs having high protein binding are not good candidate for CR formulations. Protein binding increase the biological half life as it acts as a reservoir of drug protein complex in plasma. Therefore, the need for SR/CR formulation is not required. Pharmacokinetic factors for drug selection is case of SR/CR formulation are biological half life of the drug candidate should between 2 to 8 hrs. Absolute bio-availability should be 75% or more. Steady state concentration should be lower and apparent volume of distribution should be smaller. Intrinsic absorption rate should greater than release rate. Elemination rate constant of the drug should be known. Total clearance should be dose dependent.

Patient physiology is also an important factor of dose design as patient's disease state, Gastrointestinal transit (means the time by which food leaves the stomach), residential time; GI disease can influence the release of the drug from the dosage form directly or indirectly (7).

Biological factors like absorption, uniformity in rate and extent of absorption. Other factors like solubility, log P, acid hydrolysis can affect the absorption drug. To prevent dose dumping, the rate of absorption should be rapid than release rate (7).

CHAPTER-3

OSTEOARTHRITIS(COMMON FORM OF ARTHRITIS)

Osteoarthritis (OA) is a disease cause of which is breakdown of cartilage of joint and underneath bone. (5). The Osteoarthritis means osteo bone from Greek word arthon means joint and suffice it is means inflammation.

Sign and symptoms of disease : - Most common symptom of OA is pain in joint and stiffness which means flexibility of the joints are reduced. At the beginning, these symptoms may occur following exercise thereafter with time these becomes constant. There are symptoms like swelling of joint and numbness of the arms or legs (1) in case the back is affected. The principal symptom of OA is pain resulting ability loss and reduction of flexibility. It is occur during morning and last for about half an hour. Due to pain the affected person is unable to exercise resulting muscle loss (2) (8). Joints at the base of thumb, knee, hips, neck, lower back are more commonly affected (1). Joints of one side of body generally more affected than other side (1). Normal work and daily activities are affected (1) (6). OA is thought to be caused due to loss of cartilage and underneath bone is affected (1). Mechanical stress on joint and also low grade inflammatory process is also another belief of the cause of OA (7).

Prevalence of OA: Worldwide OA is the most common form of arthritis. Total affected person in OA is approx 237 million, about 3.3% of world population are affected (4) (10). It is a disease in which elderly peoples are most commonly affected. People above 60 years of age about 10% male and 18% female are suffering from OA (2). Medical imaging helps to rule out other problems in diagnosis. Exercise is one form of treatment, efforts to decrease joint stress is anther form, support groups also help along with pain medicine (1) (3). Some people complain increase in pain in cold climate, high humidity, a drop in barometric pressure (15). In case all forms of treatments/medication fail, joint may last for 10-15 years (9).

Risk factors:- Symptoms of OA can disrupt one's normal daily life for pain and stiffness (14). Principal cause of OA is damage from mechanical stress and insufficient self repair in joints (19). Due to incorrect positioning placement of bone may cause stress, others include pathogenic cause, injury, excess body weight, muscle supporting joint is lost, impairment of peripheral nerves which can cause sudden or lack of coordination of movements (19).

Primary risk factors ;- a previous joint injury along with obesity is a major cause of OA, it causes mainly knee OA(22).

ii) More prevalent among post menopausal women than men in same age group may be due to change in sex hormone level, which is the cause of OA(23)(24).

Occupation :- people who work with manual handling like lifting of heavy objects and others with physically demanding work like climbing of stairs, ladders are affected with knee OA.

In case of hip OA, persons who work in mostly in bent or twisted position are generally affected(6). Persons who work in kneeling or squatting position are mostly affected by knee OA. So physical work like heavy lifting, coupled with kneeling or squatting position and work in standing position for long hours are mostly affected in OA. Occupational risks of developing OA are similar in men and women.

Systemic risk factor :-

a) Age ;- Age is the most common risk factor in the development of OA. Tensile property of cartilage, intra articular cartilage is lowered with age which means gyration accumulation and mechanical failure also causes with increase in age.
b) Gender;- Women are more prone to pain and disability in OA. Rate of OA in men is 58.5% and in women is 68% in age 65years or above., this was observed in a hospital based study.
c) Genetic/hormone :- Genetic factor in population causes OA, prevalence between 39% to 65% of OA in population. Monozygotic(MZ) twins with identical genes showed genetic factors in development of OA. Osteocalcin, a marker of bone turnover and increasing level of this marker i.e bone turnover is lower in women with knee OA.
d) Physical activity/ sports ;- Those women who practice gymnastic or Kung FU regularly are at risk of knee injury and may suffer from knee OA at later age. A diagram showing risk factors of OA are shown in fig.1

Secondary risk factors of OA ;-

Many pathological conditions may cause OA. Such as Alkaptonuria, congenital disorders of joints, diabetes (it is a risk factor of having joint replacement surgery at a much lower age due to OA compared to nondibetics.), Ehlers- Danlos syndrome (A group of inherited joint disorders affecting joints), Hemochromatosis (Iron overload), Wilson’s disease, injury to joints and ligaments, deterioration of ligaments.

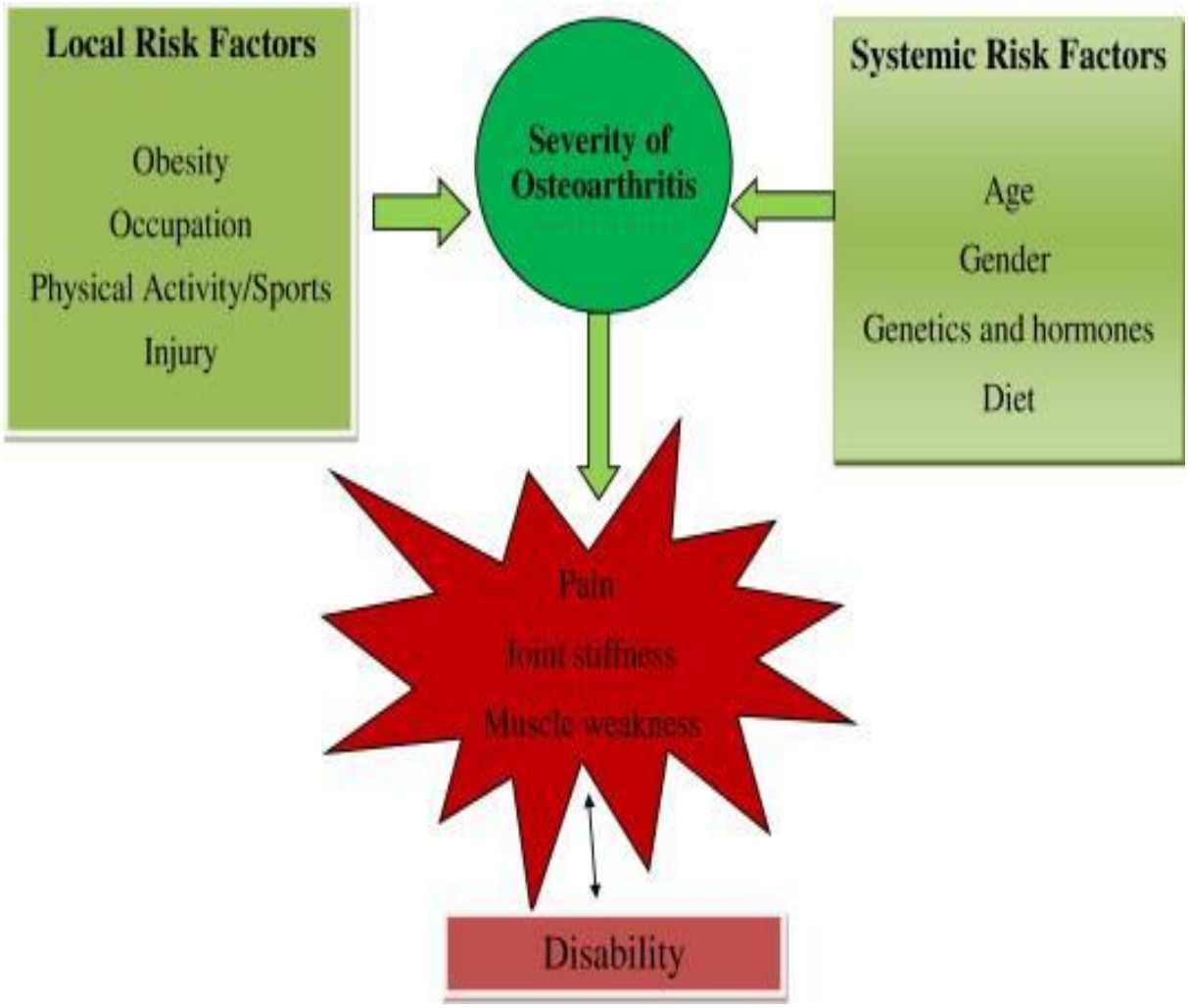


Figure 3.1.: Diagram of risk factors for osteoarthritis.(OA)

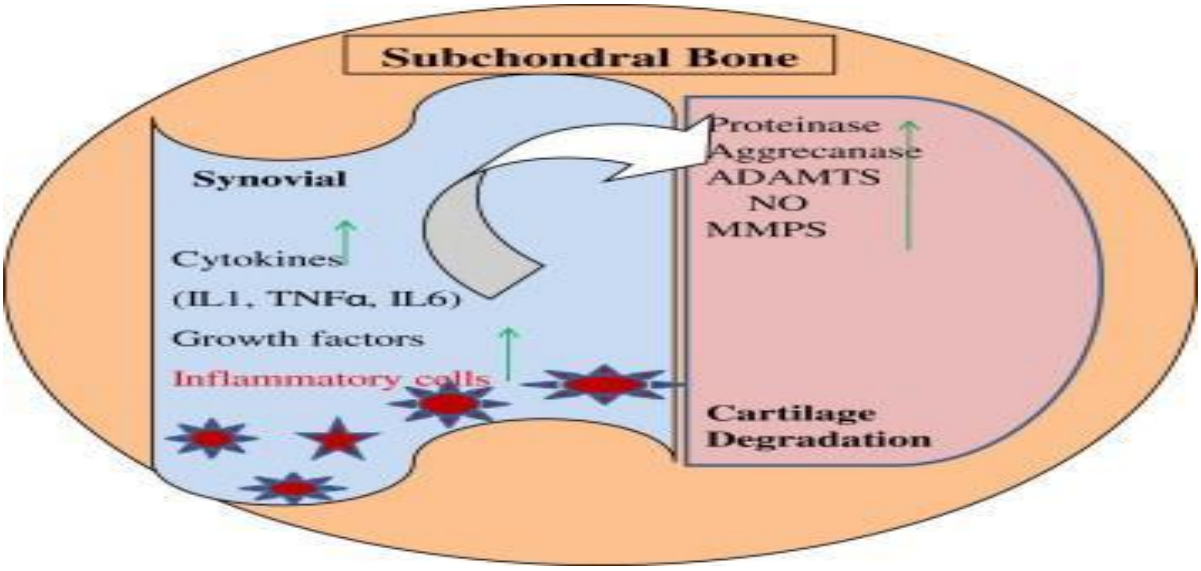


Figure 3.2: OA (pictorial representation) how it is growing up in knee joint.

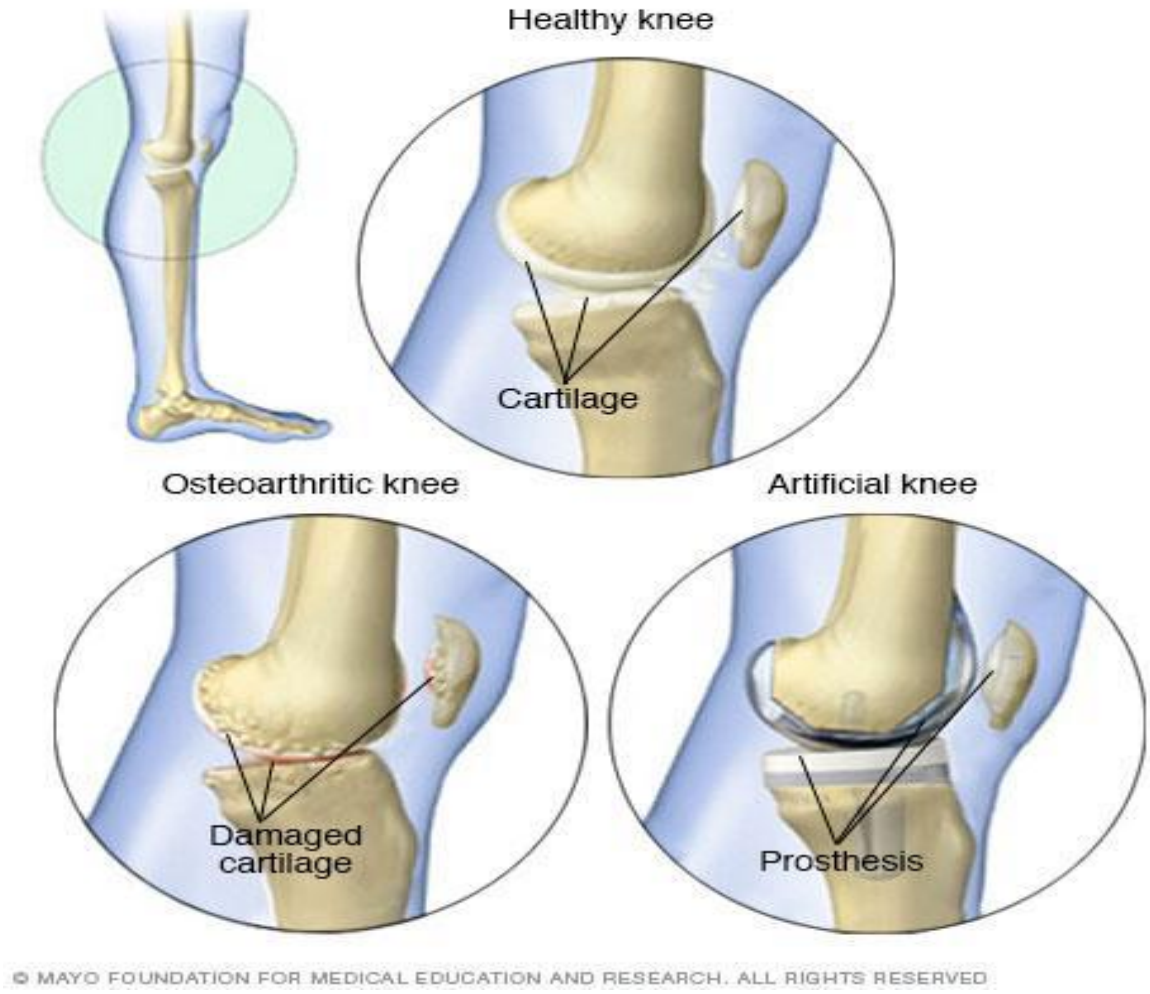


Fig. 3.3

Treatment:- *OA can't be cured, but treatments can reduce pain and help patients move better.*

Drugs which can relieve, primarily pain, and thereby improve quality of life.

Non steroidal anti-inflammatory drugs (NSAIDs).

- Acetaminophen/Paracetamol. May be of help to some people with OA who have mild to moderate pain. It can cause liver damage, if taken higher dose than the recommended dose.
- OTC analgesic drugs (NSAIDS) mainly Ibuprofen and Naproxen sodium can be taken as per recommended dose and it can reduce pain of OA. Some strong analgesics are available on production prescription. .

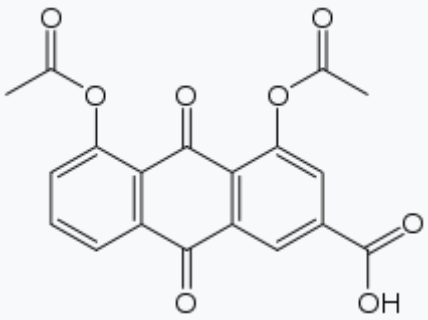
Analgesics mainly non steroidal anti-inflammatory drugs have serious adverse action on prolong use for example G.I .ulceration, cardiovascular problems, bleeding problems, and hepatic and Kidney damage. However, NSAIDs formulated as gel, for applying to the skin over the affected joint, have very less adverse effects and may give pain relief as well.

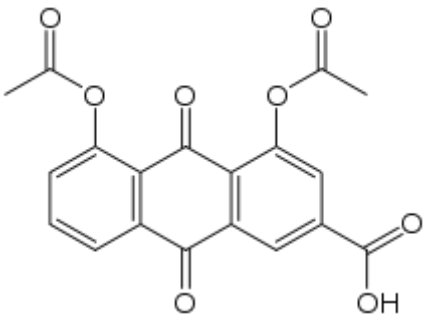
Diacerein is an anthraquinone derivative can be used in osteoarthritis as chondroprotective, it helps to reduce cartilage break down and also relieves pain and inflammation.

Duloxetine: It is therapeutically antidepressant (SSNRI) used to give relief in chronic pain. , this medication is also approved to treat chronic pain, including osteoarthritis pain. Helpful in nerve related pain and pain sensitivity.

CHAPTER-4

PHYSIOCHEMICAL PROPERTIES OF DIACEREIN





Chemical structure of Diacerein or diacetylrhein

Synonyms:- Diacetylrhein; Discerhein; (1)

Chemical Structure :- (1)

Diacerein is an anti-inflammatory drug used in arthritis and osteoarthritis. It is a drug of anthraquinone class. It is also known as diacetylrhein. **IUPAC name of Diacerein is 4,5-bis(acetyloxy)- 9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid**. Molecular formula C19H12O8, molar mass 368.294g/mole. It is practically insoluble in water, 3.197mg/litre (20^{0c})(4). Melting point of Diacerein is 218^{0C}. It is yellow colour crystalline powder, sensitive to moisture, in presence of moisture change in colour takes place, it is to be stored at room temperature 25-30 ^{0C}. It is soluble in DMSO.

Manufacturing process :- Rhein-9- anthrone -8- glycoside containing aloe emodin

↓

Components Put forward to liquid extraction

↓

1,8- dihydroxyanthrone glycosides

↓

Oxidized

↓

1,8- dihydroxyanthraquinone glycoside

↓

Glucose unit split of

↓

1,8 – dihydroxyanthraquinone compound

↓

Acetylation

↓

Diacetylrhein

i. Crystallization
ii. Dried under extremely mild condition

Diacerein

ii) **ROLE OF DIACEREIN IN OSTEOARTHRITIS** :

DCN having onset of action within 4-6 weeks and shows effect after 4-8 weeks of discontinuation of therapy. It acts by inhibition of IL-ß converting enzyme(ICE) that leads to decreased biosynthesis of IL-ß. Inhibition of biosynthesis of IL-ß by DCN leads to improvement of all pathological responses that involved in the articular cartilage destruction in OA. As DCN blocks formation of active IL-ß, the formation of IL-ß mediated expression of various proteolytic enzymes like MMP-1,MMP-3, MMP-13, ADAMTS-4 and ADAMTS-5 also reduced which leads to decreased cartilage destruction. Apart from these, DCN induced inhibition of active IL-ß biosynthesis also increases TGF-ß1 and TGF-ß2 expression that is highly essential for cartilage growth and maintenance of cartilage structure. DCN reduces the ratio of chndroitin-6-sulphate to chondroitin-4-sulphate, because it decreases chondroitin-4-sulphate depletion. Aggrecan protective effect can be seen as it mainly consists

of chondroitin sulphate and keratin sulphate. This is highly required for cartilage to counter the pressure. It is also responsible for inhibition of IL-ß mediated activation of NF-kB and activator protein-1(AP-1) transcription factors that leads to inhibition of expression of pro inflammatory cytokines, adhesion molecules, enzymes etc. DCN inhibits the binding of these transcription factors. It decreases the expression of IL-1 receptors on the surface of chondrocytes and also indirectly increases the expression of IL-1 receptor antagonist. DCN also inhibits IL-1ß mediated inducible nitric oxide synthase (iNOS) and NO production which leads to decreased chondrocyte apoptosis. During neurodegenerative and inflammatory disorders, palmitoylethanolamide (PEA) produced by mammalian cells that shows anti inflammatory and analgesic properties. PEA also down regulates degradation of mast cells and release of inflammatory mediators. PEA is metabolized to palmitic acid and ethanolamine by the enzyme N-acylethanolamine-hydrolyzing acid amidase(NAAA).DCN shows potent inhibitory effect on NAAA that leads to inhibition of inflammatory effects due to increased level of PEA. Apart from its inhibitory effect on phagocytic activity and migration on macrophages, DCN also inhibit superoxide production, phagocytic activity and chemotaxis of neutrophils. During degradation of cartilage, plasminogen activator(PA) system is proposed to play the key role in the remodeling of extracellular matrix. This system consisting of urokinase type plasminogen activator(uPA), tissue type plasminogen activator (tPa), urkinase type plasminogen activator receptor(uPAR), Plasminogen activator inhibitor-1(PAI-I).uPAI is a serine protease enzyme having molecular weight 55-kDa.It is released as inactive form as pro-uPA. Binding of pro-uPA to its receptor uPAR to uPA which responsible for conversion of plasminogen to plasmin. Increased expression of uPA can be detected in OA and rheumatoid arthritis(RA) synovial fibroblast. DCN reduces the expression of urokinase type plasminogen activator receptor(uPAR) nearly normal level and decreases the fibrinolytic activity in synovial fluid.

POSSIBLE ROLE OF DIACEREIN IN RHEUMATOID ARTHRITIS :

Numerous literature states that IL-1ß plays a crucial role in the pathogenesis of RA. It directly involved in the secretion of various photolytic enzymes like MMP-1, MMP-3 & MMP-13 and ADAMPTS which are mainly involved in cartilage destruction. IL-ß responsible for reactive oxygen species (ROS) which increases the production of superoxides like hydroxylated redicals and peroxides like neutrophils that play an important role in cartilage destruction. It is also responsible for hyperplasticity of fibroblast like synoviocytes(FLS)of intimal layer of synovial membrane that leads to the formation of

pannus and responsible for cartilage destruction. TGF-ß signaling pathway that plays significant role in cartilage growth. IL-ß shows inhibitory effect on TGF-ß pathway and prevent TGF-ß mediated cartilage growth and maintenance. IL-ß also stimulates iNOS and NO production which involved in chondrocyte apoptosis. Urokinase type plasminogen activator (uPA) and its receptor uPAR are overexpressed in the RA synovium. uPA act as a pro inhibitory mediator that having important role in the cartilage destruction.

As DCN blocks the biosynthesis pathway of active IL-1ß, it can show beneficial effect in RA by decreasing the formation of various types of MMPS and ADAMPTS as well as by decreasing the formation of superoxide by neutrophils which ultimately shows protective effect on RA cartilage. IL-1ß inhibitory effect of DCN also improves TGF-ß mediated cartilage growth. DCN shows chondroprotective effect by reducing NO mediated chondrocyte apoptosis as it inhibits IL-1ß mediated iNOS and NO production. Apart from these effects DCN also reduces the expression of IL-1 receptor antagonist. It also down regulates the expression the expression of urokinase type plasminogen activator receptor(uPAR) which decreases the fibrinolytic activity in synovial fluid. These IL-1ß inhibitory effects of DCNcan show benefial role in RA.

iii) **Pharmacokinetics of Diacerein**:-

Study shows that oral bioavailability of Diacerein is poor, about 35% to 56%. If Diacerein is taken with food, it delays physiological uptake, amount of drug absorption is increased by 25% when taken with food. Daily dose of the drug is 50mg twice daily. Conversion of the drug to rhein takes place when reaches the body. 20% of Rhein is eliminated through renal route, 60% is conjugated in liver as Rhein glucuronide and 20% is converted to Rhein sulphate. Excretion of these metabolites takes place through kidneys (2). In case of elderly people with normal renal function and young healthy volunteers have almost same pharmacokinetics action. Approx 99% plasma protein bound is Rhein, but no drug interaction takes place as the binding with plasma protein is not saturable unlike NSAIDS.

Urinary excretion pharmacokinetic parameters of Diacerein after oral administration

Parameter	Estimated value
Apparent availability of rhein by urinary data	Thirty five percent
Cmax after 50mg dose, and T_{max}	Three point two milligram per litre Two point two hour(T_{max})
Area under the Plasma Concentration time curve $(AUC)_{0-\infty}$	Twenty one point three milligram per litre per hour
Volume of distribution(Vd)	Thirteen point two litre
Total body clearance (CL_{tot})	One point six litre per hour
Kidney clearance (CL_r)	Zero point one three litre per hour
Elemination half life ($t_{½}$)	Four point three hour

iv) **Distribution** :- By double deacetylation DCN is reported to be converted to rhein. It is further converted to rhein glucornide and rhein sulphate(7)(8) (9).

v) **Elimination :-**

50mg per oral drug of DCN is eliminated through urine 19.6%, after glucoronide formation in liver rhein glucoronide 60%, rhein sulphate 20%.

Side effects:-

Diarrhea or soft stool (mostly occurs in first two weeks of the treatment)

Liver disorders (LFT abnormalities, elevated liver enzymes)

Discoloration or yellow colour urine may be due to elimination of Rhein metabolites through urine but having no clinical importance.

Skin rash , Pruritus, Eczema in some cases. (1)

Special warning ;

- Restricted to older adults (above 65 years) due to diarrhea.
- Treatment may be started on half the normal close (i.e. 50mg daily than 100mg daily).May be stopped if diarrhea occurs.

- Not prescribed to patients with liver disease..
- Mainly used in symptoms of OA of hip or knee.
- Prohibited during pregnancy and lactation as safety of the drug DCN is not assured (1)

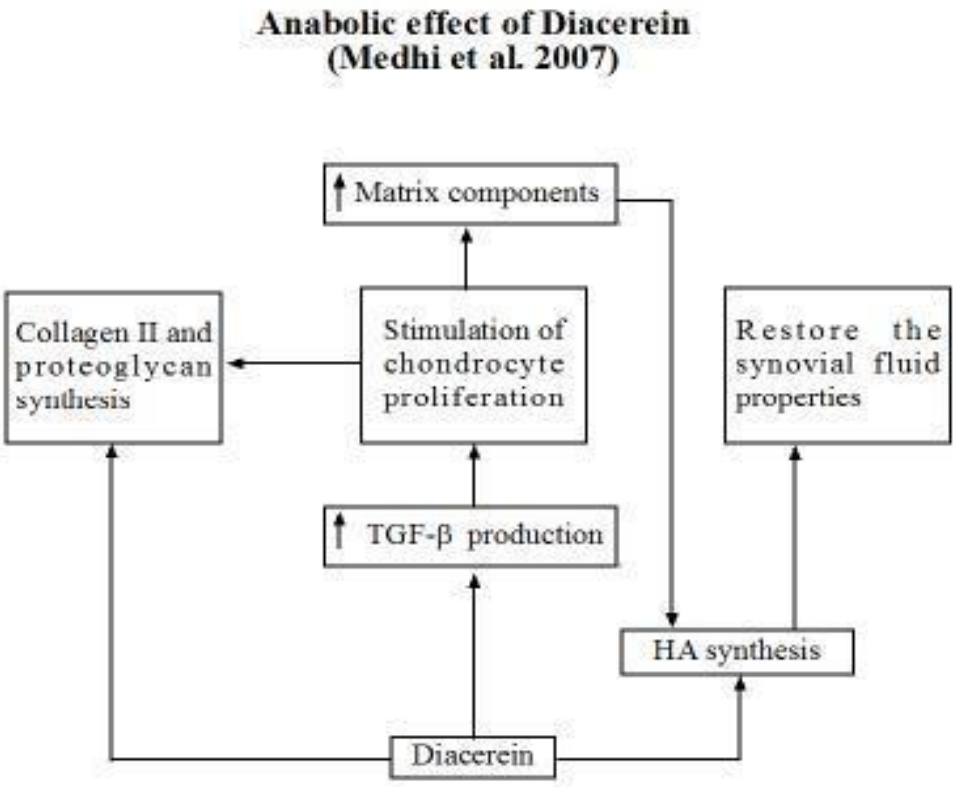
Diacerein and conventional **NSAIDS** are separate in pharmacological actions (6)

i) DCN prevents the loss of hydroxyproline and proteoglycans present in the cartilage by stimulating the production of TGF-ß. (7) (8)

ii) Diacerein has no effect of inhibiting phospholipage $-A_2$, cyclooxygenase, lipooxygenase and prostaglandins (eiconoids). Hence, DCN does not produce gastro-duodenal ulceration(Pelletier et al, 1998; Zaki et al 2013) (9) (10)

Mechanism of action:-

i) synthesis of proteoglycens which are inhibited by IL-1(Felisaz et al., 1999) (14)



DCN was first synthesized in 1980. FDA approved it as drug for osteoathritis in 2008, as it has preventive effect on I-L-1ß and I-L-6 like proteins tending to cause inflammation.

Polymer used in preparation of microsphere

HPMC 6cps (Hydroxypropyl methyl cellulose also known as methocel)

It is a taste less, bland, having no odour , nontoxic white powder. Apart from its various uses in film coating, suspending etc., it is used as hydrophilic polymer, it has wide use in manufacture of sustained /delayed release dosage form. The reasons for its use in manufacture of sustained release drug is rapid hydration and gelling characteristics. It can affect the release of drug by swelling and cross-linking. Pharmaceutical grade HPMC is available in different viscosity grade like E5, E6,E15,E4M, K100, K4M, etc. E6/ HPMC 6cps has viscosity 5.6-7.0 cps.

Ethyl cellulose (EC N7) :- (monograph available in pharmacopoeia of China, Eur. Ph and USNF)

It is an inert hydrophobic polymer widely used in number of dosage form for sustained/ delayed release drug. It is available in different viscosity grade such as N&,N10, N20, N50, N100, N200 , N300 of USP grade etc. It is also known as Ethocel and characterized by low flammability and it is also resistant to discoloration by sunlight. It is also used as binder in tablet manufacturing and coating of tablet, granules, manufacture of microparticles and as thickener. It is water insoluble, soluble in organic solvent eg. Chloroform, tetrahydrofuran etc.

Sodium Alginate (Eur. Ph, USNF 37)

It is sodium salt of alginic acid, natural polymer and it has the ability to form stable porous structure. It is used in manufacture of antacid. It has very good biocompatibility with the loaded drug for which drug release is sustained to the maximum extent and the pharmacological action of the drug remain intact . Sodium alginate based hydro gels have wide range of applications in sustained/controlled release drugs. Due to ionic character of alginates, it causes pH- dependent disintegration of the microspheres. Drug release takes place by diffusion and erosion of polymer matrix. Using sodium alginate as polymer intra nasal drug delivery can also be done due high biocompatibility.

CHAPTER-5

LITERATURE REVIEW ON MICROSPHERE FORMULATIONS

1. Ahmed A. H Abdellatif et al formulated microsphere of **Flurbiprafen** as sustained release formulation with ethlcellulose that result showed that the formulated microspheres serve as promoting platform to improve the solubility, absorption and sustained release FLB (Bull pharmsci. Assiut University, vol 39,2016,pp27-41.

2. Muserref et al investigated **Flurbiprofen** microsphere with chitosan as polymer affecting release of the drug. Method used for microsphere formulation was inotropic gelation. Chitosan solution along with FLB was added drop by drop into sodium alginate solution containing Sodium tripolyphosphste(TPP) and microspheres were formed. Microspheres were studied for drug entrapment efficiency by UV Spectrophotometer, particle size by Laser Difractometer, Surface topography by SEM, and in-vitro drug release study. (Braz. J. Pharm. Sci, vol 54, no.4, Sao Paulo, 2017, Epub, April 09, 2018

3. Naved Juaer Ayon et al prepared **Gliclazide** microsphere with cellulose as sustained release dosage form and studied different parameters like drug ingredient compatibility, particle size, drug release etc.(Dhaka Univ. J. pharm. 13(2) 149-166, 2014 (December).

4. Prasant Patil et al formulated **Flurbiprofen** microsphere sodium alginate, chitosan were used as release retarding polymer. Microsphers were prepared by ionic gelation method.Morphology by SEM, drug entrapment efficiency, particle size and shape, drug release were determined. (IJPSR, SR no 47, page 5388-5393,01Dec. 2018.

5. Venkatesh D. P. et al prepared **Fluvastatin sodium**(lipid lowering agent) which was formulated as microsphere. Drug resinate complex was formed, cholestyramine resin was used here. Ethylcelluose and eudragit R S 100 was used as polymers for sustained release formulation. All parameters like drug content, drug entrapment, particle size and shape, morphology, stability and drug release were studied (IJDDR, April-June2012, 4(2):306-3014),

6. Poonam Dhawale et al formulated **Ondansetron Hcl intranasal mucoadhesive microsphere**. This was done to avoid first pass elemination of the drug and also to

improve its residence time. Using two mucoadhesive polymers e.g. Carbopol 940 and HPMC K 15M and adopting solvent evaporation method microsphere was prepared. As film forming agent ethyl cellulose was also used.(Journal of Applied Pharmaceutical Science, vol08,pp 075-083, August 2018.)

7. Neha s Rout et al prepared buoyant microspheres **Metoprolol succinate** with ethyl cellulose and HPMC as polymers and examine influence of pH modifiers (Inst. J. Pharm. Investigation, July 2013, vol3,issue 3, pp 163-170.

8. Pritam Banerjee et al developed of **Metformin Hcl** microsphere by solvent evaporation technique. The microspheres prepared with pectin, ethyl cellulose , HPMC, and Acry coat s100 as polymer produces with sustained release properties. (Inst Schlorly Research Network, Vol 2012, article ID 230621,7 pages)

9. Paroma Arefin et al prepared **Fexofenadine** HCl microsphere using HPMC K 100 M CR as polymer as sustained release dosage form using solvent evaporation method. Formulations were found stable and reproducable. Drug loading efficiency, surface topograghy and in- vitro release were studied.(Spingerplus,2016,5(!),691,May 25 2016, online)

10. Megha Sharma et al prepared **Repaglinide** microsphere with ethyl cellulose and HPMC as polymers. The microsphere formulation was studied for in- vitro and in-vivo release patterns. The optimized formulation shows an effective way for prolong release.(Saudy Pharmaceutical Journal,2015,23,675-682.)

11. Dalgit Kaur et al carried out compatibility studies on pre and post formulation of **Diacerein** based on ATR_FTIR for the design of transfersomal studies. In preformulation study, assessment of possible incompatibility between drug and excipients is very important. (IJPSR, 2019, vol 10, Issue 5, 412-417)

12. Australian Scientists prepared new formulation of **Diacerein** to treat EBS, topical Diacerein, mainly used in treatment of osteoarthritis, hold therapeutic use in blistering in Epidermolysis Bullosa simplex type(Ablinger M, el orphaned J Rare Dis 2018).

13. Rahul P Gaikward et al worked on preparation and evaluation of sustained release floating microparticulate oral drug delivery system where **Theophylline** anhydrous

was used as drug for gastro retentive floating drug delivery system. Physical and chemical evaluation, stability were done in the study of gastro retentive sigle unit and multiple preparation.(Acta Scientific Pharmaceutical Sciences 3.5(2019):128-141, vol 3, Issue 5, 5th May 2019)

14. A Agarwal et al prepared **Chitosan** microspheres by an aqueous process and drug release was studied, pages 819-823, published online 29 Sept. 2008, Journal of Microencapsulation, Micro and Nano carriers. vol. 18, 2001-Issue 6..

15. Swapna S et al. prepared flowting microspheres of **Diacerein** by ionic gelation technique. HPMC (K 100M) and sodium alginate were used as polymers and sodium bicarbonate as gas generating agent and calcium chloride as cross-linking agent. Purpose was to maintain the dosage form flowting in stomach for controlled and known release of drug and to enhance bioavailability. (wjpls 2018, vol. 4, Issue 7, 117-123).

16. Gomez-Gaste C et al and others worked on biodegradable PLGA microparticle (MPS) of **Rhein** for intra-articular administration of rhein. Preparation of MPS was done by emulsion-solvent evaporation technique. (Eur. J. Pharm Sci. 2017 Jan 1; 96; 390-397)

17. R.D Kale and P.T. Tayade et al prepared **Piroxicam** microsphere as a floating drug delivery system of Piroxicam. where Eudragit S was used as polymer. Microspheres were prepared by solvent evaporation method and showed significant delayed release action. (Indian J Pharm Sci., 2007, 69(1); 120-123).

18. Phutane P et al and others prepared sustained release microspheres of **Glipizide**, an anti diabetic drug. The objective of the study was to reduce dosing frequency and improve patient compliance. Ethyl Cellulose was used as control release polymer and microspheres were developed by emulsion solvent diffusions evaporation technique by using modified ethanol-dichloro-methane co-solvent system. (J. Young Pharm 2010. Jan 2; (1): 35-41)

19. EL-Setouhy DA et al. formulated **Leflunomide** microsphere, a drug used in Rheumatoid Arthritis. Microspheres were prepared in a manner to be used as intra articular injection which would also produce sustained drug release effect. PDLG polymers (Lactide/glycolide copolymer) and poly capro lactone (PLC) at two drug: Polymer ratios (1:2 and 1:4) were used. Advantage with them that they are

biodegradable. Polyvinyl alcohol or HPMC was stabilizers in solvent evaporation method adopted for manufacture of microsphere. (Shodhganga inflibnet. ac. in Shah P.A, review of literature)

20. Venkateson P et al. formulate and evaluate **loxoprofen** a NSAID as sustained release microsphere by solvent evaporation technique using Ethyl cellulose as sustained release polymer. Studies like particle size, drug loading, and in vitro drug release were carried out and microspheres showed 71.2% of entrapment and in vitro drug release study showed that Loxoprofen microspheres of 1:3 drug polymer ratio showed better sustained release effect.. (Published in Pulsus, www.pulsus.com, available online 15-08-2011).

CHAPTER-6

MATERIALS & METHOD & PROCESS OF MANUFACTURE

Materials used in the preparation of Diacerein microsphere:-

i) Diacerein ph. Eur.:- Mfg. by :- Elder pharmaceuticals Ltd, Patalganga, Mumbai (free gift).
ii) EC N7:- -S.D fine chemicals.
iii) Hydroxy propyl methyl cellulose 6 cps – Do
iv) Microcrytaline cellulose pellets: - Pharmatrans Sahao.
v) Isopropyl Alcohol: - Merck India Ltd. Mumbai.
vi) Methylene Dichloride: - Nutan chemicals Pune, Maharashtra.
vii) Dibutyl Phthalate: - Labdhi chemicals Mulund west Mumbai.
viii) Sodium alginate, Star chemicals

Reagents & chemicals used in the study are of AR grade

Name of the Instruments /Equipment:-

* Mini Lab Coater, Manufacturer- Umnag Pharmatech Pvt. Ltd. Moharasshtra
* Homogenizer RQ-127A REMI MOTOR
* Digital Electronics Balance PB 303 Delta range
  Metler Toledo
* UV Spectrometer M5 SPECTRAMAX
* HPLC – Perkin Elmer (Model Flexar)
* In-vitro dissolution tester, Make :- Lab India, model DS 8000+
* FTIR – Shimadzu (Model Prestige 21)
* DSC ;- (Model DSC 4000, software Pyris)
* SEM Model :-JSM6360, JEOL MAKE
* X-Ray Diffractrometer ULTIMA-III RIGAKU MAKE ,JAPAN

Manufacturing process of Diacetylrhein Microsphere by Spray coating (wurster method)

First Stage: -:Drug loading was done on MCC pallets (300gm size #18-20,1mm- 0841mm) and primary coating was done with hydroxy propyl methyl cellulose 6cps

Table 6.1: Formula for coating solution

Sr. No.	Ingredients	Quantity (%)	Quantity (g)
1.	Diacetylrhein	Five point three four%	Hundred ten g.
2.	HPMC 6 cps	Two point four three%	Fifty g.
3.	Isopropyl alcohol (50%)	Forty six point one two%	Nine fifty g.
4.	Methylene Dichloride	Forty six point one two%	Nine fifty g.
	Total	Hundred %	Two zero six zero g.

Restoration: 7.77% w/w

Method of manufacture :-

- Mixed 110.0 g. of Diacetylrhein and 50.0 g. of HPMC 6 cps and passed through # 40mesh.
- The above blend was added to Isopropyl Alcohol under stirring. Methylene dichloride was added to the blend and continued stirring for 45 minutes.
- Passed the above coating solution through # 80 mesh muslin cloth.
- Stirring was continued during the entire coating process to avoid settling.

Different parameters of coating:

Table 6.2

Channel(Inlet) Temperature	Forty degree centregrade
Yield (Product) Temperature	Thirty one to thirty five degree centregrade
Outwear (Exhaust) Temperature	Twenty eight to thirty two degree
Flow of air (cfm)	Six to eight
Commute(Drive (%)	Forty five to fifty five
Dissipation(Atomization (bar)	One point five to one point seven
Spray pump (rpm)	One to four
Process pump (rpm)	One to four
Process time	Six hours

Increase in weight (theoretical): 36.67 %

Increase in weight (actual): 35.00 %

Second Stage: Sustained/ control release coating

Weight of Drug loaded pellets used for coating: 405.0 g

Formula for coating solution:

Table 6.3

Sr. No.	Ingredients	Quantity %	Quantity (g)
1	Ethyl cellulose N7	Four point nine five	Fifty g
2	Dibutyl phthalate	Zero point nine nine	Ten g
3	Isopropyl Alcohol	Forty seven point zero three	Four seven five g
4	Methylene Di chloride	Forty seven point zero three	Four seven five g

Restoration : 5.94 % w/w

Preparation method :

- Mixed 50.0 g of Ethyl cellulose N7 and 10.0 g of Dibutyl phthalate.
- The above mixture was added to Isopropyl alcohol under stirring weighed quantity of Methylene Dichloride was added to this blend and continued stirring for 45 minutes.
- Passed the above coating solution through 80 mesh muslin cloth.

Coating parameters:

Table 6.4

Channel (Inlet) Temperature	Forty degree centregrade
Yield (Product) Temperature	Thirty one to thirty five degree centregrade
Outwear (Exhaust) Temperature	Thirty one to thirty two degree centregrade
Flow of air (cfm)	Seven to eight
Commute (Drive (%)	Fifty five to sixty five
Dissipation(atomization (bar)	One to one point nine
Spray pump (rpm)	One to five
Process time	Four hours

Increase in weight (theoretical) : 14.81%

Increase in weight (actual) 13.58 %

Weight of drug and excipients = 460 gm

By spray coating method manufactured microspheres were uniform spherical in size and further studies were carried out for its characterization in later part of the thesis.

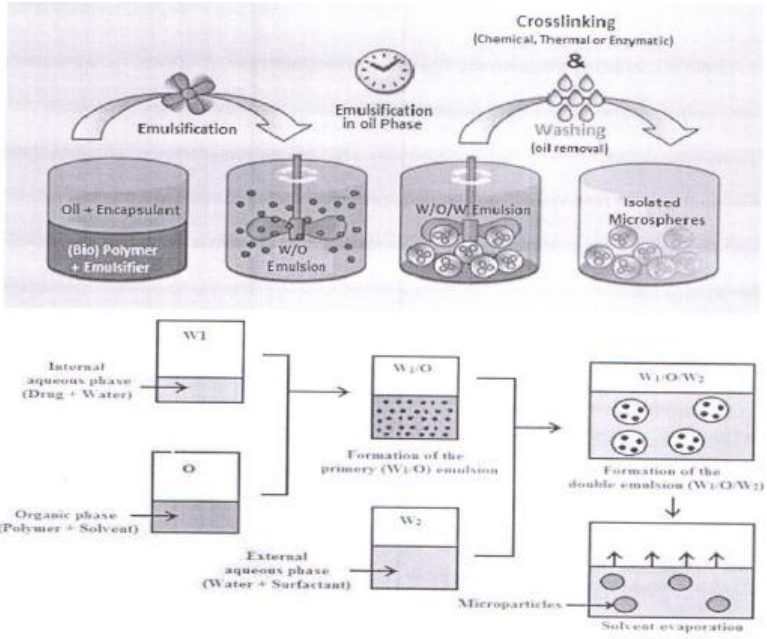
Manufacture of Diacerein microsphere by Double emulsion method :-

Primary water in oil (w/o) emulsion was prepared with surfactant of low HLB value and secondary emulsification was done with surfactant of high HLB value to form w/o/w double emulsion. Here w/o/w double emulsion was prepared with internal and external aqueous phase generated by oil layer to ease of formation and stabilization In double emulsion solvent evaporation method, an aqueous solution or suspension of sodium alginate with Diacerein (aqueous phase) was added under stirring with ethyl cellulose in dichloromethane (oil Phase) using 0.2ml span 80 in a homogenizer (Remi motor, model 127a, Mumbai) in 5000rpm and duration 5 min.

Table 6.5

Parameters	Process condition
DCN : Sodium-alginate: ethyl cellulose	(1:1:12), (1:3:24),(1;5:15)
Surfactant (Span 80)	0.2ml
Surfactant(Tween80)	0.2ml
Dichloromethane	20ml
Stirring speed Water	5000 rpm 100ml

Resulting water-in-oil (w/o) emulsion was added to100ml distilled water containing 0.2ml tween 80 with continuous stirring (Type BL-433, REMI Motor) at room temperature to form double emulsion. Thereafter, evaporation of dichloromethane, the organic solvent was done by stirring speed of motor was set to 700 rpm and continued for 3hrs. After solvent evaporation the polymer precipitated and the core of the microsphere solidified. Then the microspheres were separated by passing through muslin cloth washed with cold distilled water and dried at room temperature for 24 hrs.



The schematic representation for the method of preparation is shown below:

Water + Natural polymer from plant gum exudates

↓

Stirring for 30minutes at 30-35^{0c}

↓

Suspension (**W1**)

↓

Polymer (ethyl cellulose) + Dichloromethane + Span80 {oil phase **O1**}

↓

Stirring few minutes and **W1** added drop
by drop with Homogenization

Primary Emulsion (**W1/O1**)

↓

Distilled water + Tween 80 (**W2**)

↓

(W1/O1) is taken in a 12 no. syringe added
Drop by drop by continuous mechanical
Stirring at 600-700 r.p.m for 5-6 hrs

Microsphere formation in solution by evaporation of the solvent oil

↓

Microsphere was separated by filtration & Washed, Collected & Dried

\

Formulation of Diacerein microspheres containing sodium-alginate with different ratio of Diacerein and sodium-alginate and ethyl-cellulose :

Table 6.6

Formulation	Drug polymer ratio(mg)	Amount of drug (mg)	Amount of Sodium Alginate(mg)	Amount of Ethyl cellulose(mg)	Stirring speed
F1	1:1:12	30	30	360	5000
F2	1:5:15	30	150	450	5000
F3	1:3:24	30	90	720	5000

1) **Percentage yield of microsphere by Double emulsion method**:-

After the preparation of microsphere, they are accurately weighed and the yield % was calculated :-:

Yield %=Total amount of DCN microsphere manufactured ÷Theoretical amount × 100

Characterization of microspheres:-

2) Drug entrapment efficiency

30 mg of DCN microsphere was weighed accurately, powdered and poured into 30 ml of pH 6.8 phosphate buffer and stirred under magnetic stirrer for 5hr. Thereafter, the mixture was passed through Whatman filter paper and filtered portion was diluted with phosphate buffer of pH 6.8, analysed by UV spectrophotometer (Spectromax M 5) at wavelength 260 nm.

. The % entrapment efficiency of the matrix was calculated as

. Entrapment efficiency = (Drug loading ÷ theoretical drug loading) × 100

Results were based on triplicate determination.

3) Scanning Electron Microscopy (SEM)

Morphology of DCN microsphere was determined by scanning electron microscope (SEM). Scanning Electron Microscope (JEOL MAKE, MODELJSM6360 LV) was used to know the surface region and near surface region, in short the morphology of the microspheres. Experiment was done in voltage 17kV, magnification 35, 60, 75, 100, 120 times, samples with gold sputter coating were used.

4) FT-IR Spectroscopy :-

FT-IR analysis measures the IR region of electromagnetic radiation spectrum having longer wavelength and lower frequency than visible light. It is measurable in case of sample submitted to IR radiation. FTIR Can identify unknown compound, purity of a compound, consistency.

It was used in the study as to whether any interaction took place between drug and polymer. Spectra for for DCN API and DCN microsphere and DCN and ingredients physical mixture were studied in FT-IR (Prestige 21 model) The samples were prepared in KBr dises (2mg sample in KBr 200mg) Region of scanning 4000 - 400 cm-1 (mid infrared region).For analysis polymer powder was completely dried in vacuum desiccators. The samples were mounted on the sample compartment and the radiation from I.R source was collimated by a beam-splitter and thus 50% of the beam passed to fixed mirror and the other50% was reflected to the moving mirror, two beams recombined at the beam splitter after reflection and emerging beam passed through the sample compartment and focused on the detector. The signal is called inter-ferogram and have all the information required to know spectrum properly via a mathematical equation known as Fourier Transformation.

RESULT AND DISCUSSION

Double emulsion solvent evaporation method of preparation of microsphere is an easy method of preparation of sustained release dosage form, but here the drug entrapment efficiency can not be increased further, as a result its industrial application is very limited. DCN microspheres were further characterized as follows :-

5) **CHARACTERIZATION OF THE DRUG:**

The gifted sample of Diacerein was characterized by physical observations, by melting point determination, and recording of ultraviolet- visible and Infrared absorption spectra further was compared and matched with reference spectra cited in International Pharmacopoeia.

i) Physical observation: Physical observation reveals that Diacerein is fine yellow crystalline powder.

ii) Melting point determination: M.Pof the drug sample was found 217- 218°C which was matched with the literature assuring that the identity of the received sample.

iii) Ultra-violet (UV) absorption spectra: The sample of Diacerein was scanned spectrophotometrically in the range of 200-400 nm wavelengths in phosphate buffer pH 6.8.

Absorbance maxima (λmax) was found at 254-260 nm wavelength, which was used for quantitative analysis.

iv) Infrared (IR) spectra: IR spectra of Diacerein was matched with that of the reference.

Spectra in BP, and it showed similar peak.

v) Calibration curve of Diacerein:

Calibration curve of Diacerein in phosphate buffer pH 6.8 was prepared. Increase of concentration was done in a preset manner. For quantitative estimation of the drug Regression equation was calculated and utilized. The correlation coefficient was found to be 0.9972.

Data for preparation of calibration curve was shown below.

Table 6.7: Data for the preparation of calibration curve of Diacerein (mean ± SD, n=3)

Concentration (mcg/ml)	Absorbance (nm)
0	0.00
2	0.092
4	0.206
6	0.283
8	0.378

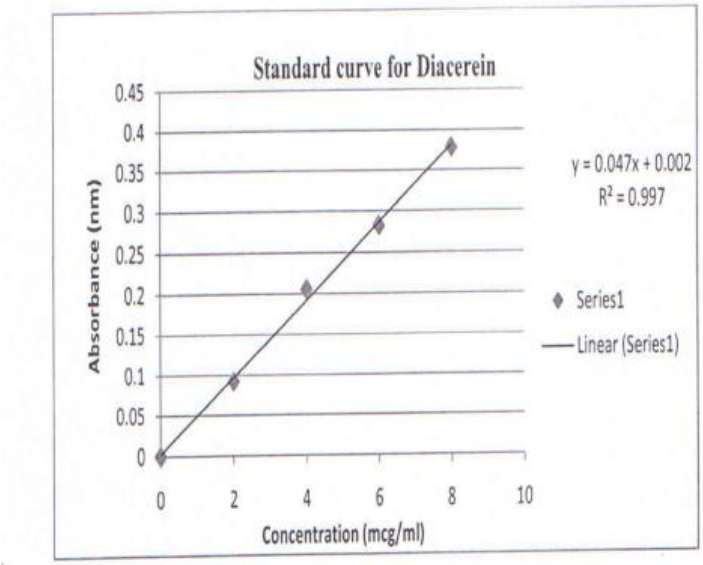


Fig6: Calibration curve of Diacerein in phosphate buffer 6.8

5.2 PERCENT YIELD AND DRUG ENTRAPMENT EFFICIENCY:

The microspheres were prepared by double emulsion solvent evaporation (W/O/W) method with different drug to polymer ratio. So the influence of different polymer concentration on Diacerein loaded microsphere was evaluated and yields were found from the range of 81-84%.

Table 6.8: Percentage yield and entrapment efficiency of Diacerein by W/O/W emulsion medthod

Formulation code and Drug: sodium-alginate: ethyl - cellulose ratio	% yield (mean ± SD, n=3)	Entrapment efficiency (%) (mean ± SD, n=3)
F1 (1:1:12)	84.52 ± 2.9	30.82 ± 2.1
F2 (1:5:15)	82.86 ± 3.6	32.18 ± 2.4
F3 (1:3: 24)	81.43 ± 3.2	39.40 ± 1.6

The drug entrapment efficiency of Diacerein in micro-sphere was determined in phosphate buffer pH 6.8 and it was found that drug entrapment efficiency for various formulations was found to vary between 31.00 % to 39.00 %. Increase in polymer concentration leads to the formation of larger microspheres, entrapping greater amount of drug, however entrapment increases with increase in drug to polymer ratio. The low entrapment may be due to partitioning out of the slightly water soluble drug from the aqueous dispersed phase into the external aqueous phase. Since dichloromethane has a limited solubility in water, it prolonged the solvent swollen conditions which involved a slower precipitation of polymer and consequently increased the drug leakage into the external aqueous phase. Another reason may be of such lowering of drug entrapment efficiency; is the value of oil-water partition coefficient, the higher partition coefficient of the drug might be responsible for such lowering of drug entrapment efficiency[45].

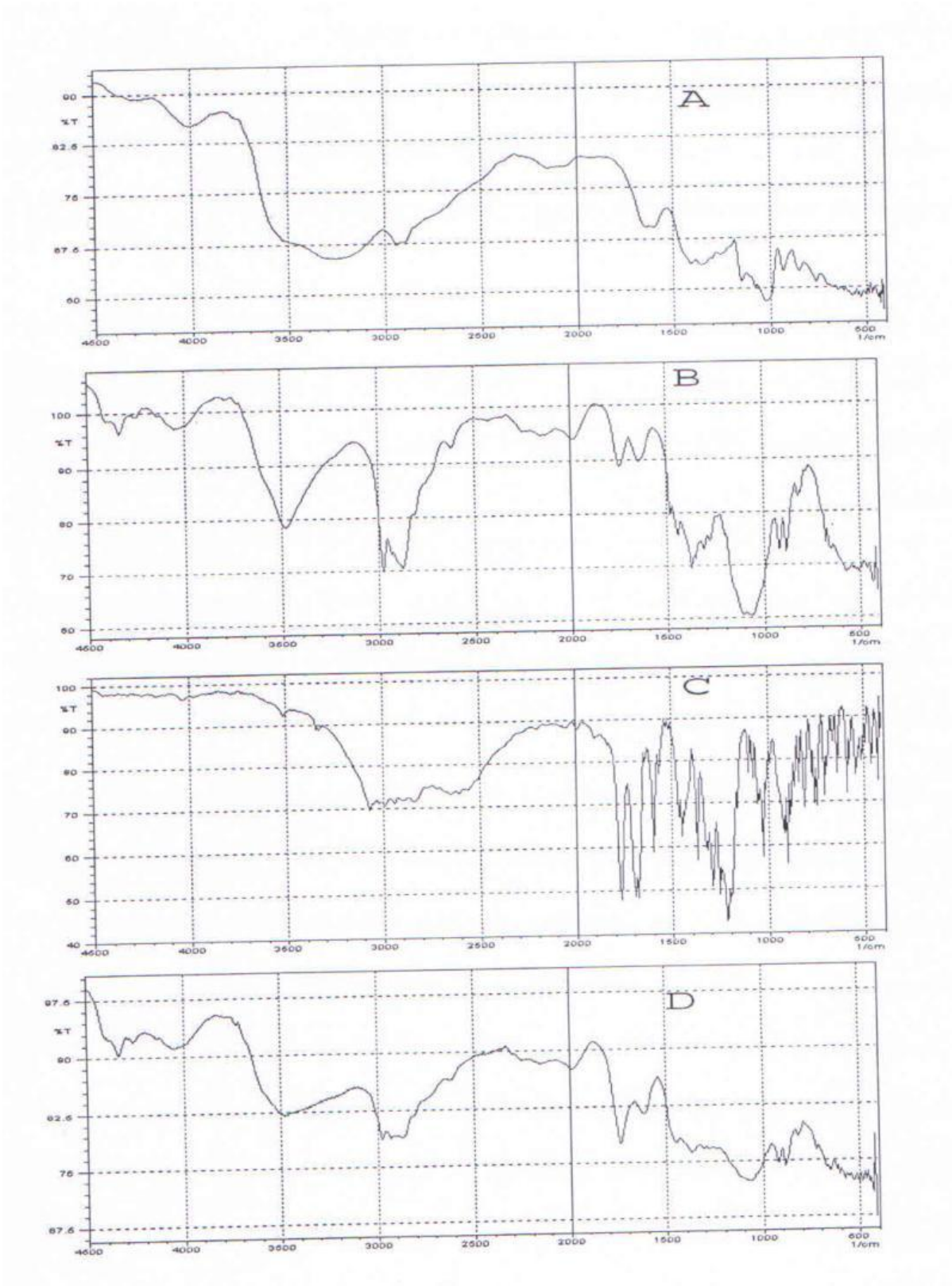
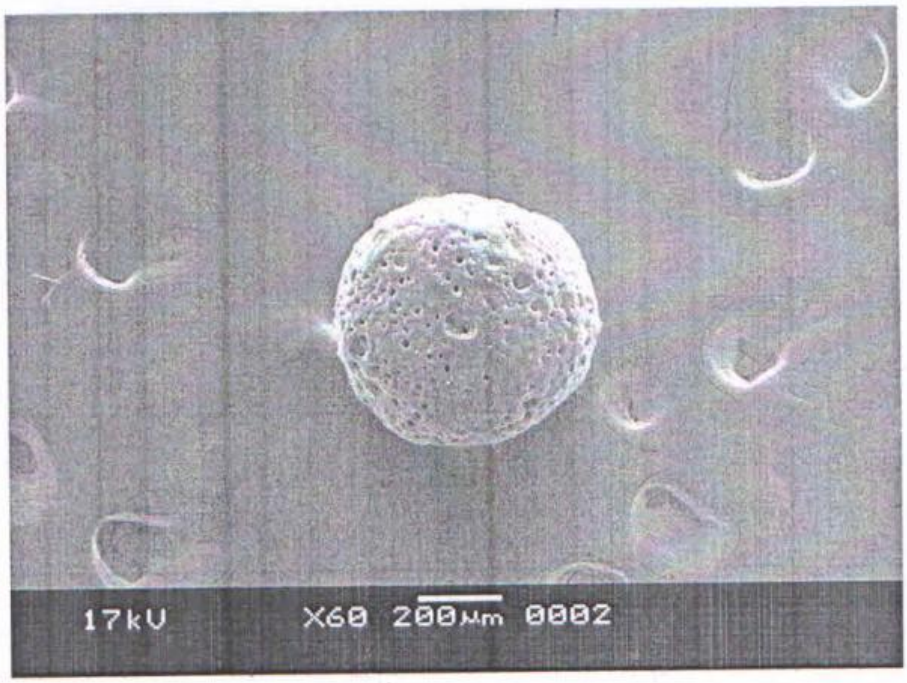
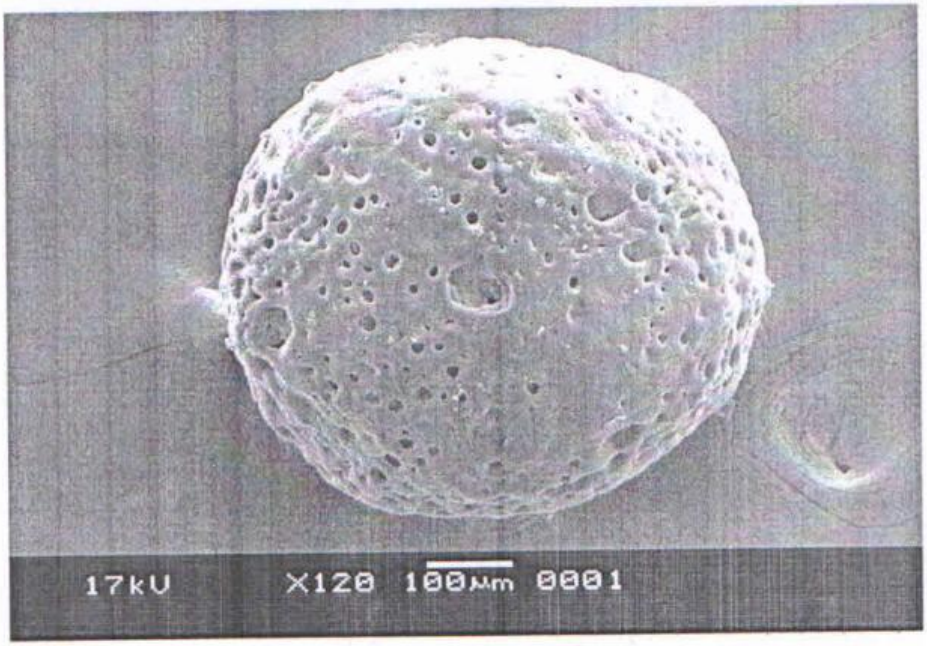


Figure 6.1: FT-IR Spectrum of (a) sodium-algianate, (b) ethyl-cellulose, (c) pure drug Diacerein, (d) combination of sodium-alginate, ethyl-cellulose, and drug Diacerein



(× 60 magnification)

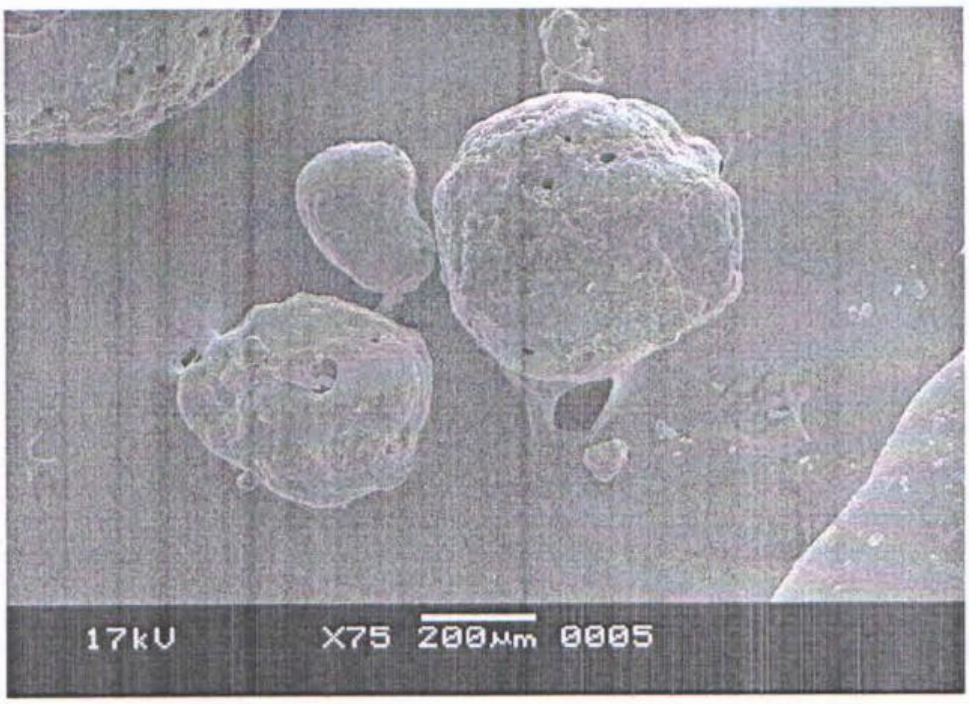
(a)



(× 120 magnification)

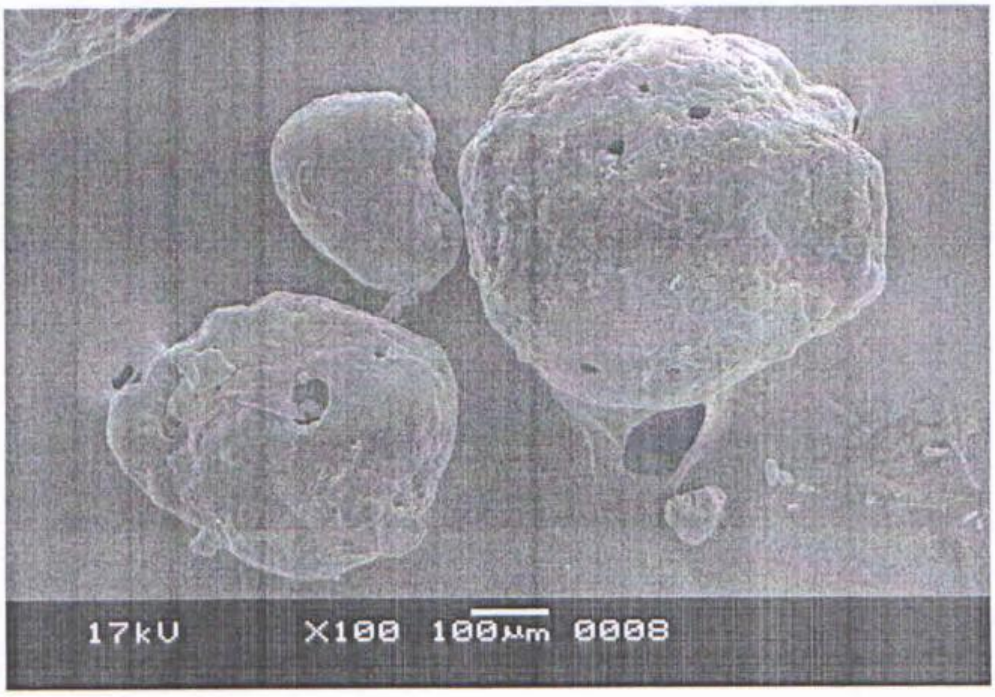
(b)

Figure 6.2: SEM photograph of Diacerein loaded microsphere (formulation F1)



(× 75 magnification)

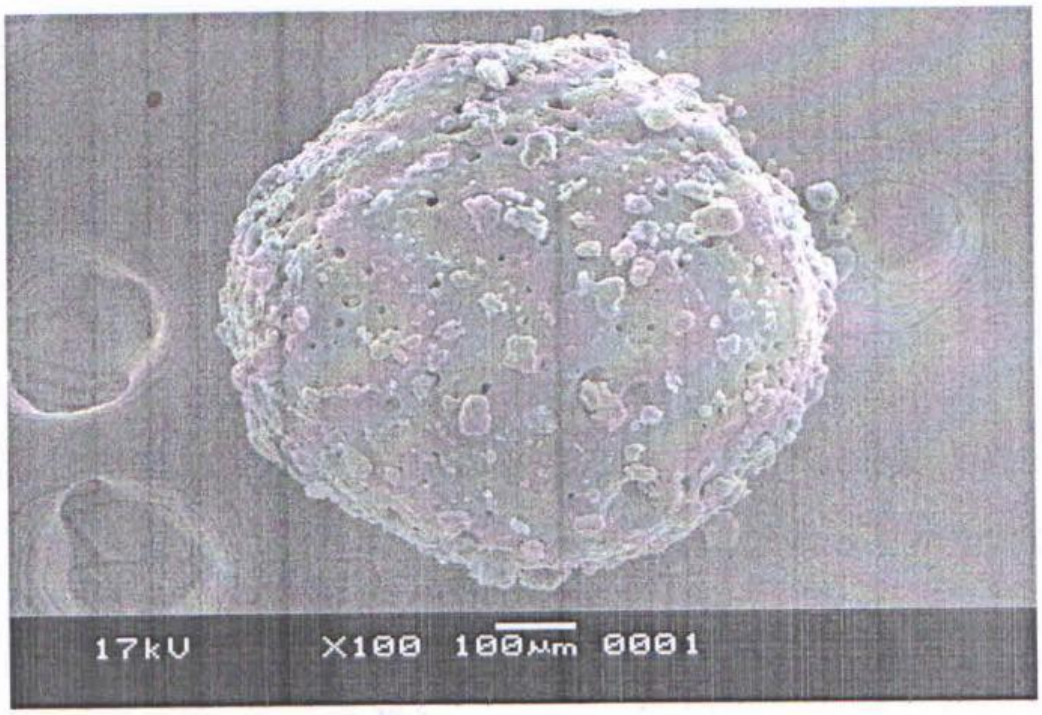
(a)



(× 100 magnification)

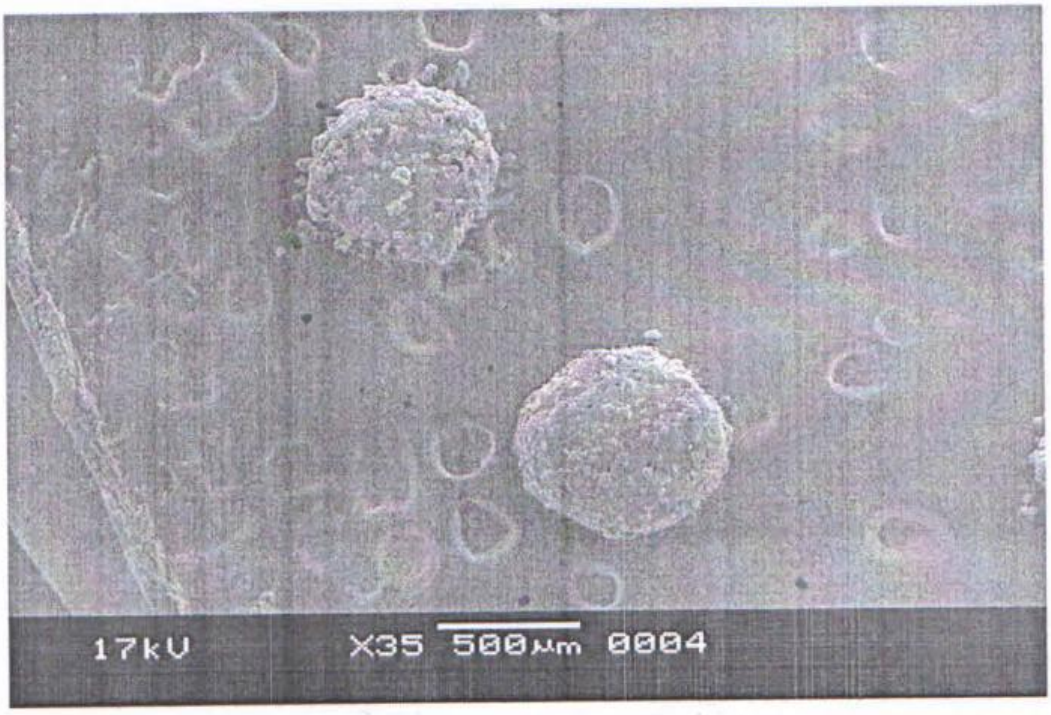
(b)

Figure 6.3: SEM photograph of Diacerein loaded microsphere (formulation F3)



(× 100 magnification)

(a)



(× 35 magnification)

(b)

Figure 6.4: SEM photograph of Diacerein loaded microsphere (formulation F2)

5.2 X-RD ANALYSIS (XRD spectroscopy):

X-RD technique is used to determine crystallographic structure of a material. It works on the principle of irradiating a material with Xrays and thereafter the intensities and scattering angles of xrays which leaves material are determined. Based on the diffraction pattern material is identified It can identify the phase of the material, XRD can also determine the deviation of material from ideal one.

It was done to establish the stability and compatibility of the drugs with polymers and with other excipients when formulated into microspheres. Since each diffraction pattern is a characteristic of a specific crystalline lattice for a given compound, the purity and stability of the drug in the dosage form can be established through it. The XRD is done at 5-70° 20. The diffraction pattern of the pure drug was compared with that of the formulations and established their characteristics.

The X-Ray diffraction pattern shows that ethyl cellulose in combination with sodium alginate do not show any diffraction pattern so it is most probably exist in amorphous form. But the formulation shows a little characteristic diffraction pattern of the drugs. Slight shift had taken place due to the reduction of purity of the drugs during formulation into microspheres. The method which is followed during the preparation may also affect the diffraction pattern.

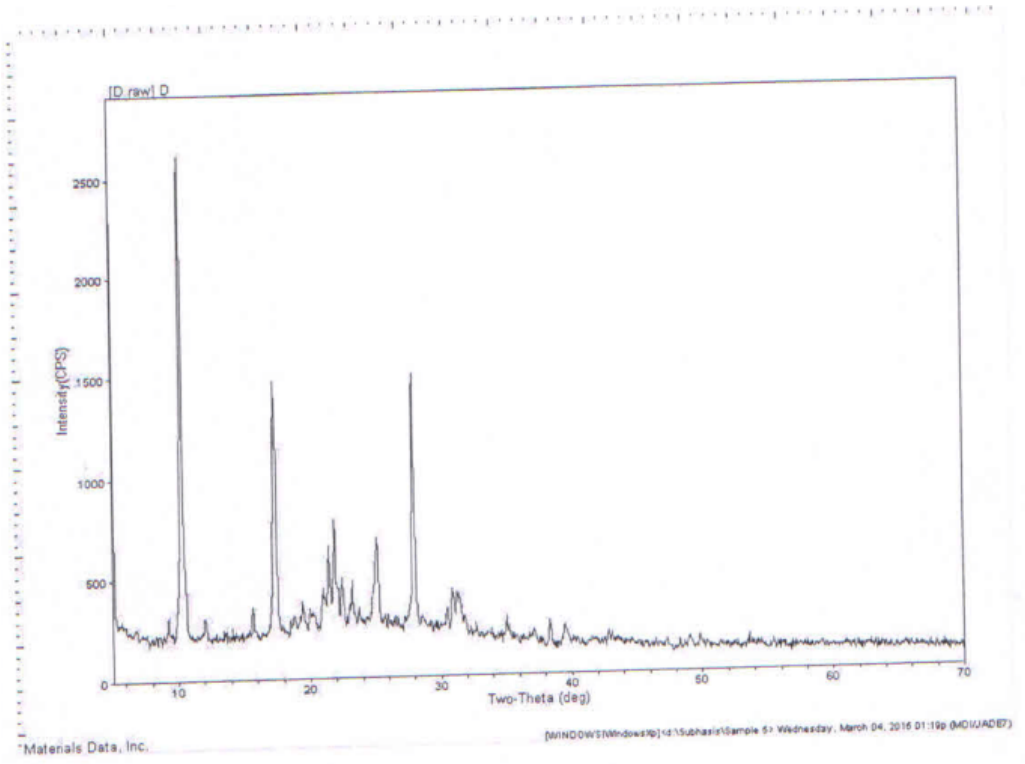


Figure 6.5: (a) X-RD Spectra of pure drug Diacerein

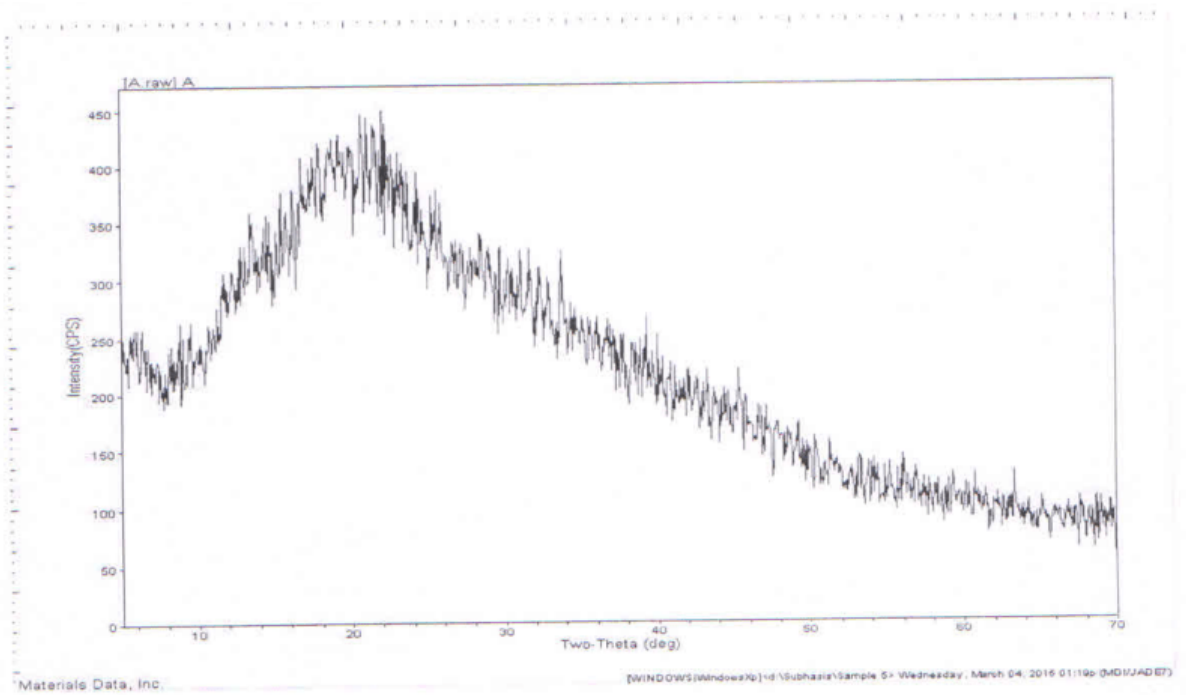


Figure 6.6: (b) X-RD Spectra of Sodium alginate

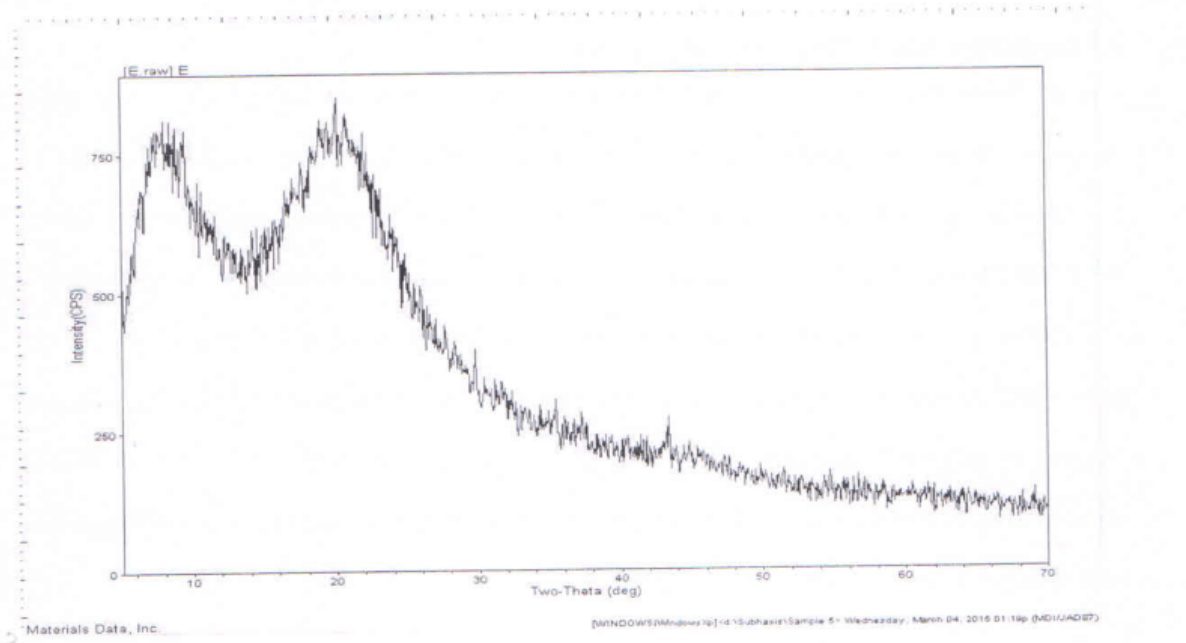


Figure 6.7: (c) X-RD Spectra of Ethyl cellulose

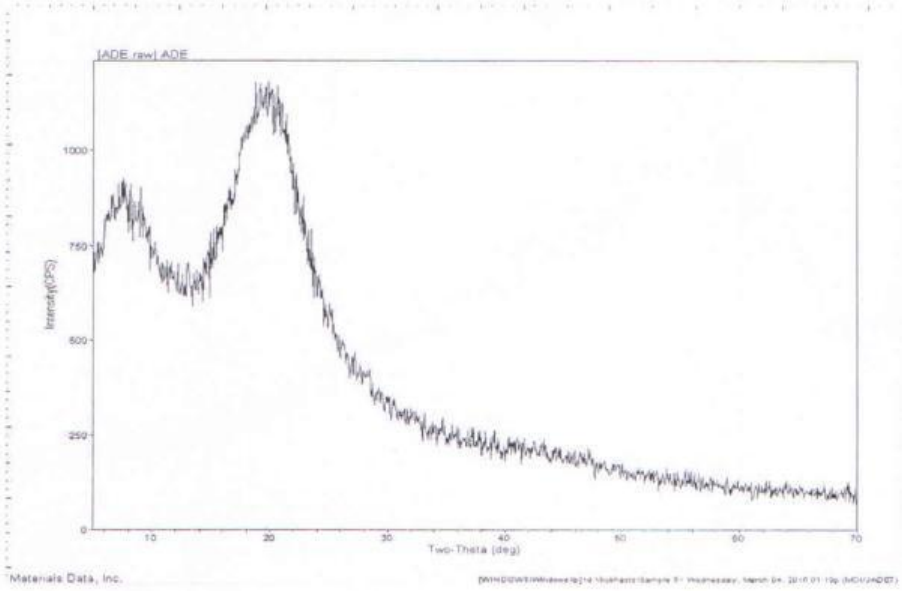


Figure 6.8: (d) XRD Spectra of combination of drug, ethyl cellulose and sodium alginate microsphere

5.6 IN-VITRO DRUG RELEASE BEHAVIOUR:

In-vitro dissolution studies were performed for all the formulations using USP type II tablet dissolution tester employing paddle type in phosphate buffer pH 6.8 dissolution medium for 11 hr. The samples withdrawn were analyzed by using UV spectrophotometer. For the different three formulations (F1, F2, F3) it has been observed the initial burst release after 30 min which was followed by a slow and continuous manner. It has been found that the drug release is very slow which decrease with the increase in drug to polymer ratio (shown in the following table and figures). A polymer's ability to retard the drug release rate is related to its viscosity. Higher alginate viscosity slowed down drug release rate in the buffer phase. The results showed that sodium alginate matrices can sustain drug release for 11 hr.

Table 6.9: Percent drug release for formulation F1

Time(hr)	% release (Mean ± SD, n=3)	AUC
0	0	0
0.5	12.04 ± 1.05	3.00906
1	13.87 ± 1.22	6.47603
1.5	16.48 ± 1.01	7.58738
2	17.00 ± 1.09	8.36403
3	22.83 ± 1.50	19.9037
5	28.91 ± 1.32	51.7489
7	29.80 ± 2.01	58.7231
9	31.33 ± 1.77	61.1395
11	31.62 ± 1.94	62.9546

Table 6.10: Percent drug release for formulation F2

Time(hr)	% release, (Mean ± SD, n=3)	AUC
0	0	0
0.5	7.82 ± 1.67	2.069
1	9.83 ± 1.34	4.52775
1.5	12.66 ± 1.08	5.62409
2	13.68 ± 1.22	6.58556
3	15.73 ± 1.12	14.7096
5	18.39 ± 1.98	34.1346
7	23.94 ± 1.09	42.339
9	25.38 ± 1.44	49.3249
11	26.47 ± 1.78	51.8582

Table 6.11: Percent drug release for formulation F3

Time(hr)	% release, (Mean ± SD, n=3)	AUC
0	0	0
0.5	14.80 ± 1.89	3.70138
1	15.60 ± 1.06	7.60367
1.5	16.73 ± 2.20	8.08676
2	18.21 ± 1.34	8.73717
3	20.58 ± 1.09	19.3958
5	21.89 ± 1.88	42.4793
7	25.80 ± 1.07	47.7011
9	27.32 ± 1.56	53.1270
11	28.53 ± 1.67	55.8447

The initial burst effect may be attributed as a desired effect to ensure initial therapeutic plasma Concentrations of drug. The drug release was decreased with increase in concentration of the polymer as increase in the polymer solution viscosity has produced microspheres with reduced porosity due to the thickening of the polymer wall. It is understood that higher polymer concentration results in a longer diffusional path length, so drug release is extended. The thick polymeric barrier slows the entry of surrounding dissolution medium in to the microspheres and hence less quantity of drug leaches out from the polymer matrices of the microspheres exhibiting extended release. Drug release profile of different formulation (F1, F2, F3) of mixed polymeric matrix microsphere provide linear relationship for Higuchi & Korsmeyer-Peppas model.

The difference in drug release behavior suggested structural difference of wall materials.

Release kinetics:

Drugs release from sodium-alginate and ethyl-cellulose microsphere is found to be triphasic release profile, first initial burst release followed by diffusion for certain time then sustained release. In search of best fit mathematical model for formulations, the analysis of dissolution data was done applying the Korsemeyer- Peppas and equation. which is shown as

$Mt/M\infty = K_{kp}.t^n$

Where, Mt = quantity of drug release at time t and M∞ =amount release at time t=∞, thus Mt/M∞ = fraction of drug released at time t, K_{kp} =Korsemeyer release rate constant, K = kinetic constant, and n =drug release exponent . Co relation coefficient r^2 value for different kinetic models were examined in different drug polymer ratio .r^2value in Higuchi and Korsmeyer –Peppas model were found hightest.The different kinetic models for different formulation are shown in figure 12, 13, & 14.

Table 6.12: Correlation coefficient (R^2) for drug to polymer ration 1:13, 1:20, 1:27 (Prepared by w/o/w method) after fitting of dissolution data to the different kinetic methods

Formulation code	Drug-polymer ratio	Kinetic model				
		Zero-order	First order	Higuchi	Korsmeyer-Peppas	
		R^2	R^2	R^2	R^2	n
F1	1:1:12	0.7716	0.8764	0.9433	0.9670	0.3519
F2	1:5:15	0.8633	0.9597	0.9826	0.9885	0.3936
F3	1:3:24	0.6980	0.9708	0.9889	0.9666	0.2289

The goodness of the fit is shown through determination of correlation co-efficient. Very high correlation co-efficient (close to 1) indicates that the release can be characterized by that model. The diffusion exponent, n was found to be between0.2 to 0.5 which indicates that drug release from microsphere is diffusion controlled.

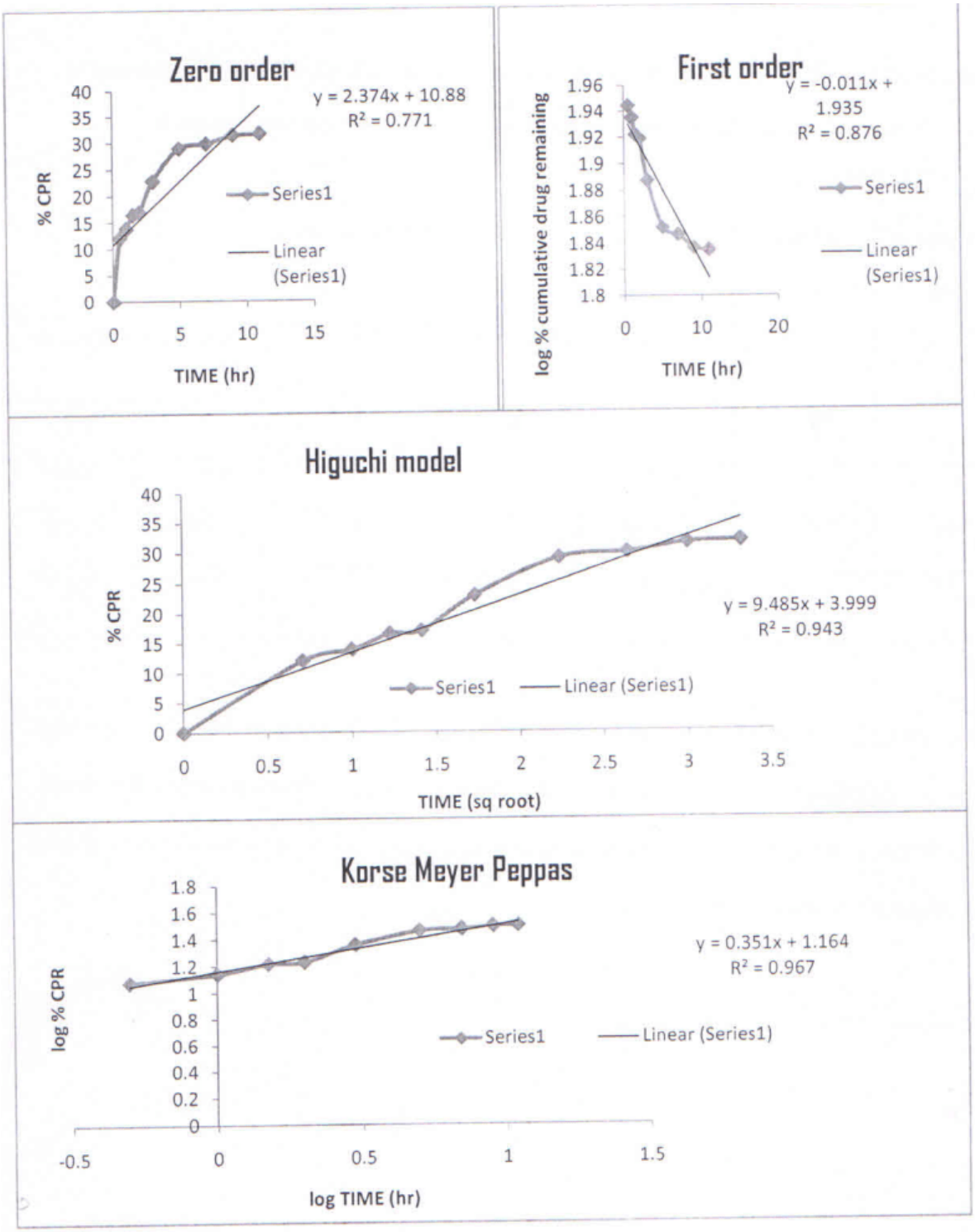


Figure 6.9: Different kinetic model for formulation F1 (1:1:12) Prepared by w/o/w emulsion solvent evaporation method

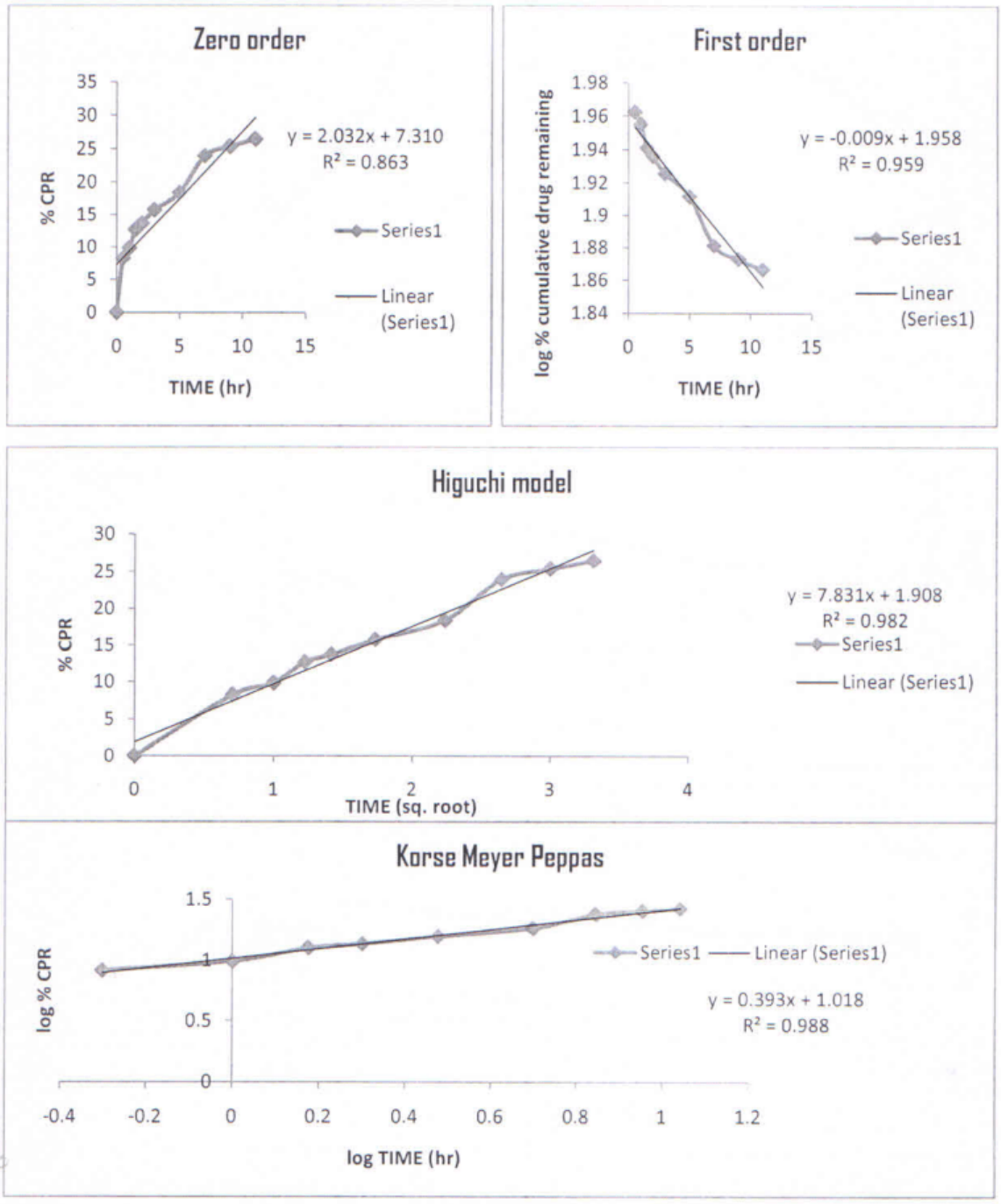


Figure 6.10: Different kinetic model for formulation F2 (1:5:15) Prepared by w/o/w emulsion solvent evaporation method

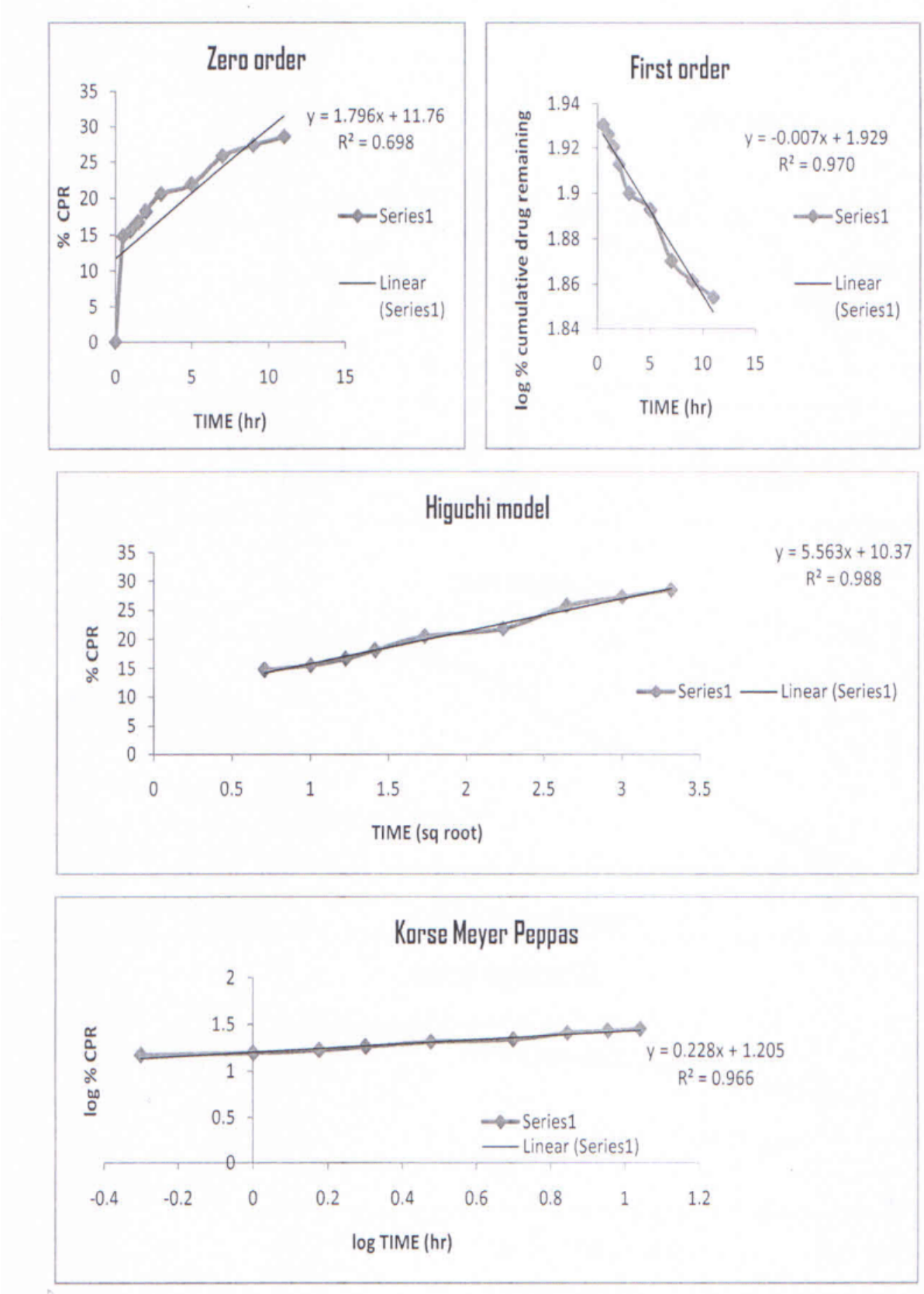


Figure 6.11: Different kinetic model for formulation F3 (1:3:24) Prepared by w/o/w emulsion solvent evaporation method

Further studies on Diacerein microsphere manufactured by spray coating method:-

Microsphere manufactured by double emulsion method produces microspheres of low drug entrapment efficiency though % yield are good in all three drug polymer ratios. Hence it has been decided to progress further with the microspheres manufactured by spray coating (wurster method). All other studies enumerated here are made of by Spray coating method.

A. Evaluation of Diacerein microsphere:

DCN microsphere assay by HPLC

The Diacerein microsphere was assayed by HPLC following method described for assay of Diacerein capsule in IP 2014 monograph with some modification. The assay was done on a C18 (15cm x 4.6mm) column with Tri ethyl amine buffer (PH 3.0 adjustment using othophosphoric acid) and Acetonitrile in the ratio 75:25 as mobile phase at a flow rate of 1.0ml/min with 20 microlitre of load. The column was maintained at 40^0C temperature and detection was done by UV detector at 254 nm. HPLC (Perkin Elmer model Flexor) was used in the assay. The microsphere formulation was finely ground in mortar pestle and accurately weighed 0.035g of pellet was taken in triplicate in 3 separate 100ml volumetric flasks and 70ml solvent mixture was added.

Solvent mixture:- 50 volumes of buffer PH 3.0 prepared by diluting 1.4ml of tri ethyl amine in 100ml of water, adjusted to PH 3.0 with orthophesphoric acid and 50 volume of Acetonitrile.

Sonication of each solution was done for 15 min and volume was made up to the mark with solvent mixture.

Further 5ml of each solution was diluted to 10ml with solvent mixture and these solutions were assayed against Diacerein standard solution of 0.05mg/ml.

Assay of DCN was calculated to be 146.17mg/gm of microsphere.

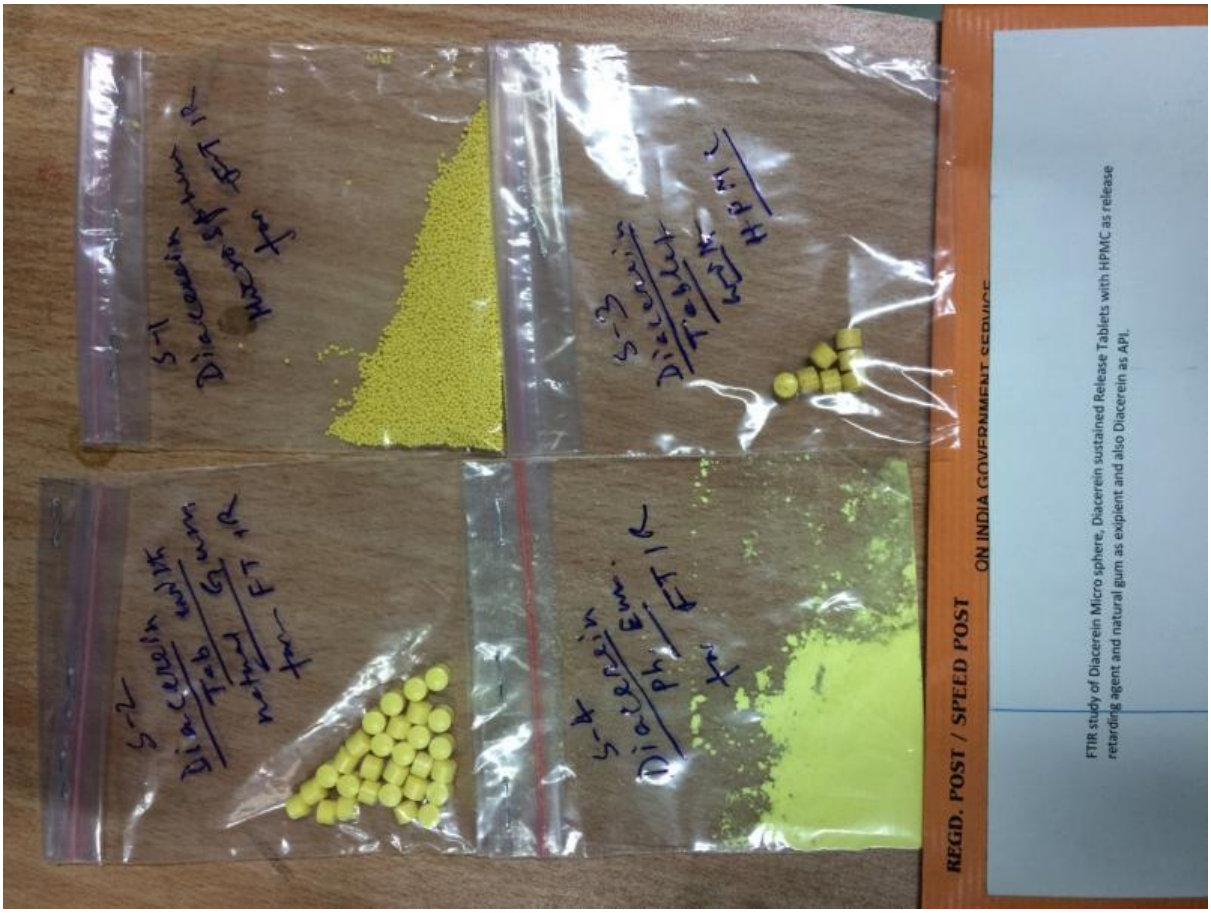
B. Drug Entrapment efficiency:-

The definition of drug entrapment efficacy is the ratio of the amount of drug encapsulated in the microsphere with theoretical drug present in the microsphere. Entrapment efficacy is determined by the formula : **drug entrapment efficacy % = (drug content determined by experiment)/(drug content theoretical) x 100**

Weight of Diacerein used in spray coating = 110g ,Total weight of drug and excipients = 460g, Theoretical drug content =110/460=0.2391g of drug/gm of material

HPLC Assay= 0.146g/g of microsphere

Drug entrapment efficiency(%) = 0.146/0.2391 x 100 = 61.06 %



Diacerein nicrosphere

CHAPTER-7

C. SCANNING ELECTRON MICROSCOPE (SEM) STUDY OF DIACEREIN MICROSPHERES (MANUFACTURED BY SPRAY COATING METHOD)

Morphology of DCN microspheres were studied by SEM method using JEISS make (UK) model (JSM 6360 LV) to scan sample of DCN microsphere with focused electron beam to images with information about samples shape and topography. Drug containing microspheres were mounted on conducting stubs and vacuum coated gold palladium film using a Gold Sputter Coater (Model Edwards-S 150 B; Mfg. BOC Edwards UK). Images were taken using 17 kv electron intensity in SEM to examine the surface morphology.

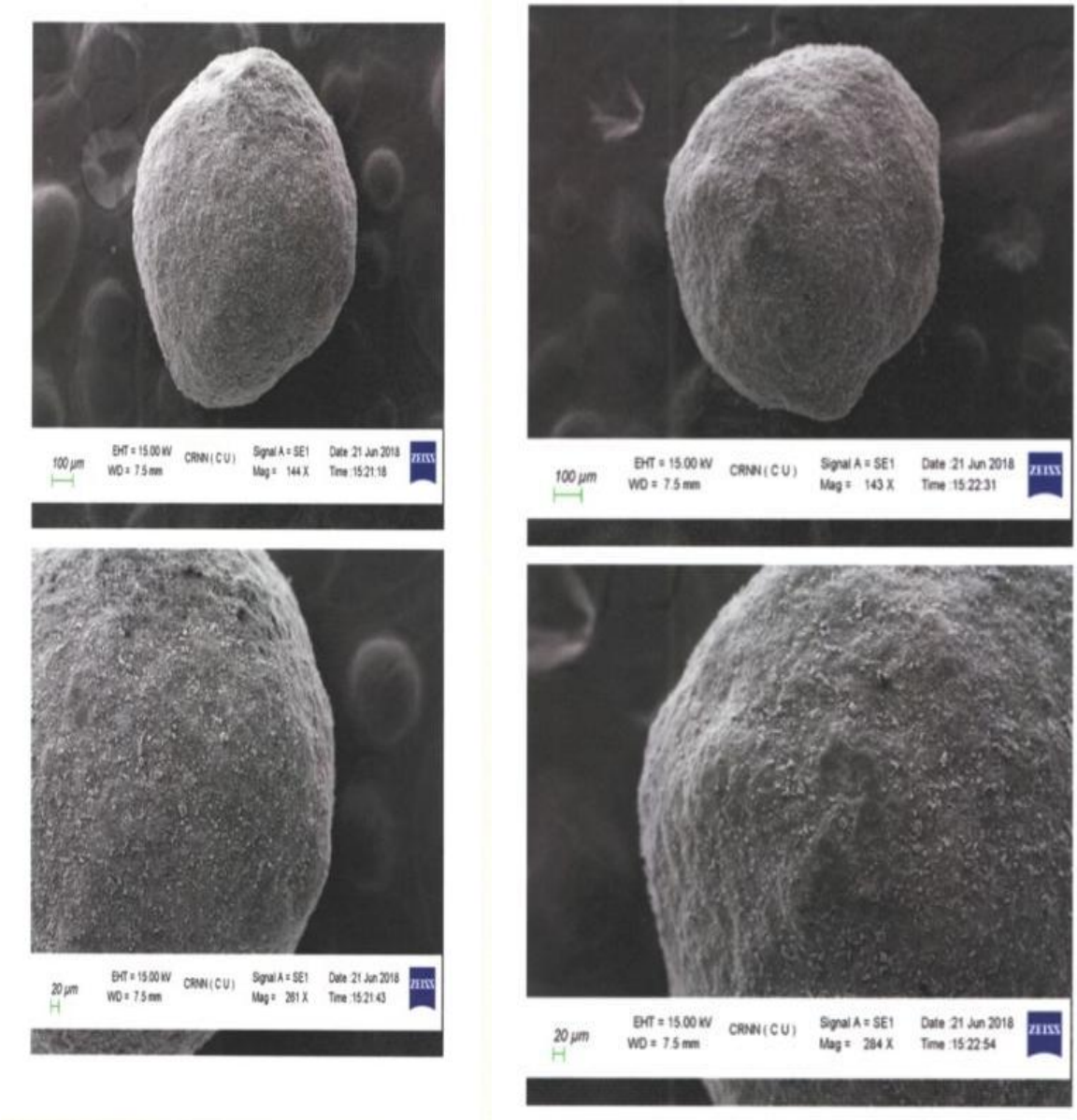
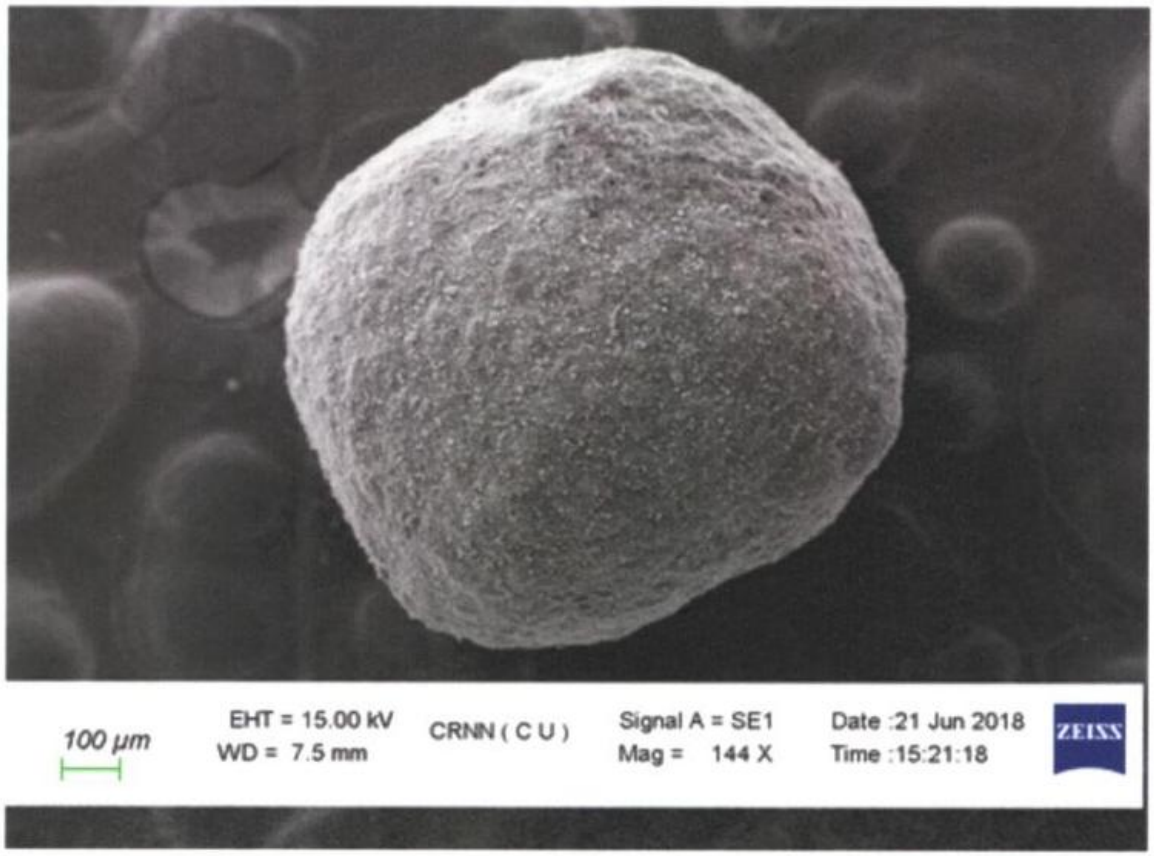


Figure 7.1-4


100 µm
EHT = 15.00 kV
WD = 7.5 mm
CRNN (C U)
Signal A = SE1
Mag = 144 X
Date :21 Jun 2018
Time :15:21:18
ZEISS

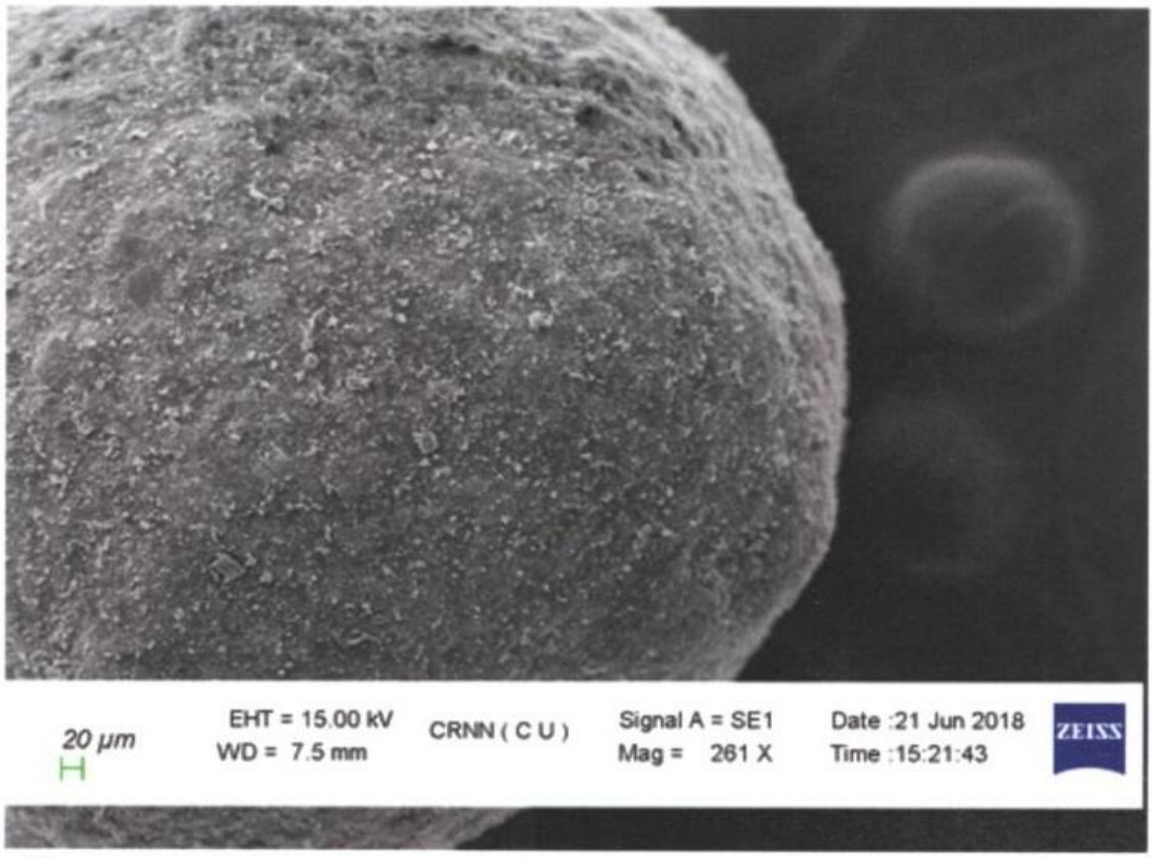

20 µm
EHT = 15.00 kV
WD = 7.5 mm
CRNN (C U)
Signal A = SE1
Mag = 261 X
Date :21 Jun 2018
Time :15:21:43
ZEISS

Figure 7.5-6

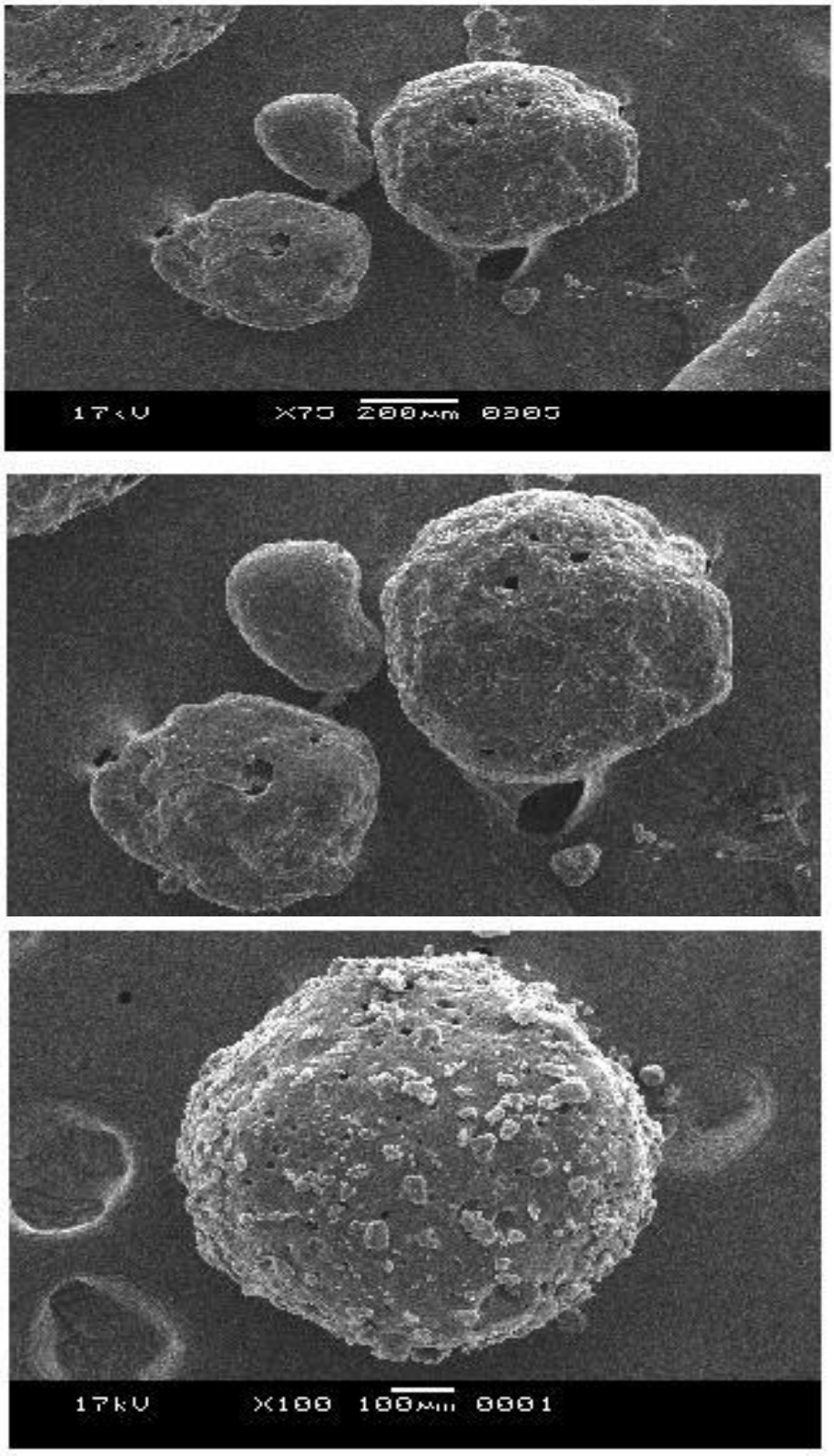


Figure 7.7-9: Diacerein microsphere by double emulsion method

CHAPTER-8

D.DETERMINATION OF PARTICLE SIZE, SIZE DISTRIBUTION. ZETA POTENTIAL AND POLY DISPERSITY INDEX (PDI) OF DIACEREIN MICROSPHERE

The particle size of Diacerein microsphere was measured by dynamic light scattering technique using Zetasizer ver.7.11, Serial Number MAL 1053771. Malvern Instruments Ltd.

Sample preparation

25 mg sample was dissolved in 25ml of Mili Q water. Thereafter 1ml of the solution was diluted to 10ml with Mili Q water and filtered with whatman 90mm filter paper. 25mg sample +H_2O (Mili Q) -25ml-1ml-1-H_2O-10ml. 4mm He-Ne laser beam of 633^0 wavelength (Zeta sizer, Malvern Instruments). A minimum of three different sets were examined at 25^0C with a detection angle of 173^0 using NIBS technology.

Table 8.1 Measurement of particle size of Diacerein microsphere

Size	**Mean**	**Std Dev**	**Size**	**Mean**	**Std Dev**	**Size**	**Mean**	**Std Dev**	**Size**	**Mean**	**Std Dev**
d.nm	**Intensity percent**	**Intensity percent**	**d.nm**	**Intensity percent**	**Intensity percent**	**d.nm**	**Intensity percent**	**Intensity percent**	**d.nm**	**Intensity percent**	**Intensity percent**
0.4	0		5.615	0		78.82	0		1106	0	
0.4632	0		6.503	0		91.28	0		1281	0	
0.5365	0		7.531	0		105.7	0		1484	0	
0.6213	0		8.721	0		122.4	0		1718	0	
0.7195	0		10.1	0		141.8	0		1990	0	
0.8332	0		11.7	0		164.2	0		2305	0	
0.9649	0		13.54	0		190.1	0		2669	0	
1.117	0		15.69	0		220.2	0		3091	0	
1.294	0		18.17	0		255	0		3580	0	
1.499	0		21.04	0		295.3	0		4145	0	
1.736	0		24.36	0		342	0		4801	0	
2.01	0		28.21	0		396.1	16		5560	0	
2.328	0		32.67	0		458.7	3.1		6439	0	
2.696	0		37.84	0		531.2	33.8		7456	0	
3.122	0		43.82	0		615.1	17.1		8635	0	
3.615	0		50.75	0		712.4	0		10000	0	
4.187	0		58.77	0		825	0				
4.849	0		68.06	0		955.4	0				

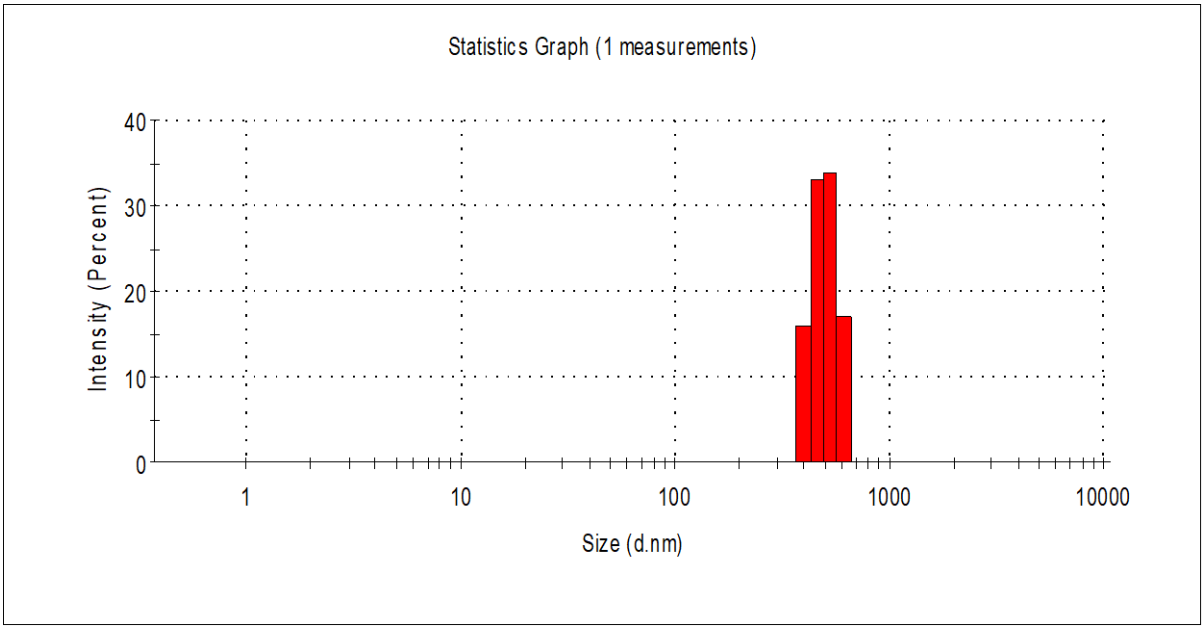


Figure-8.1

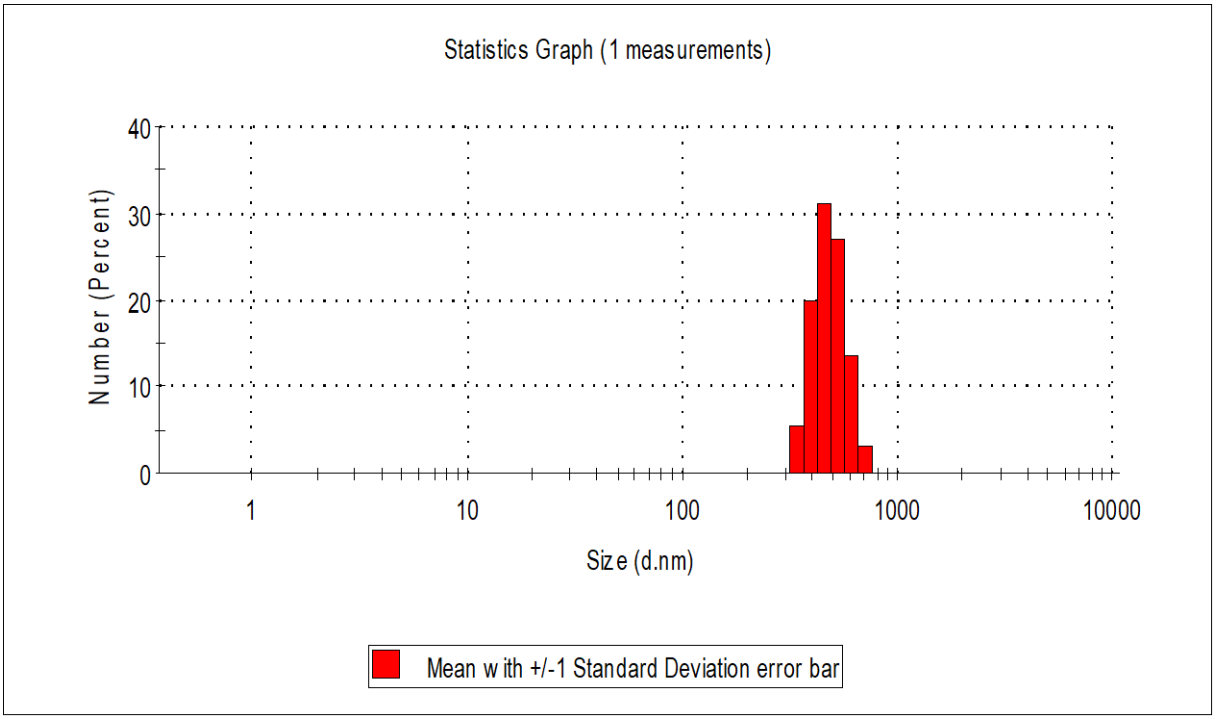


Figure 8.2

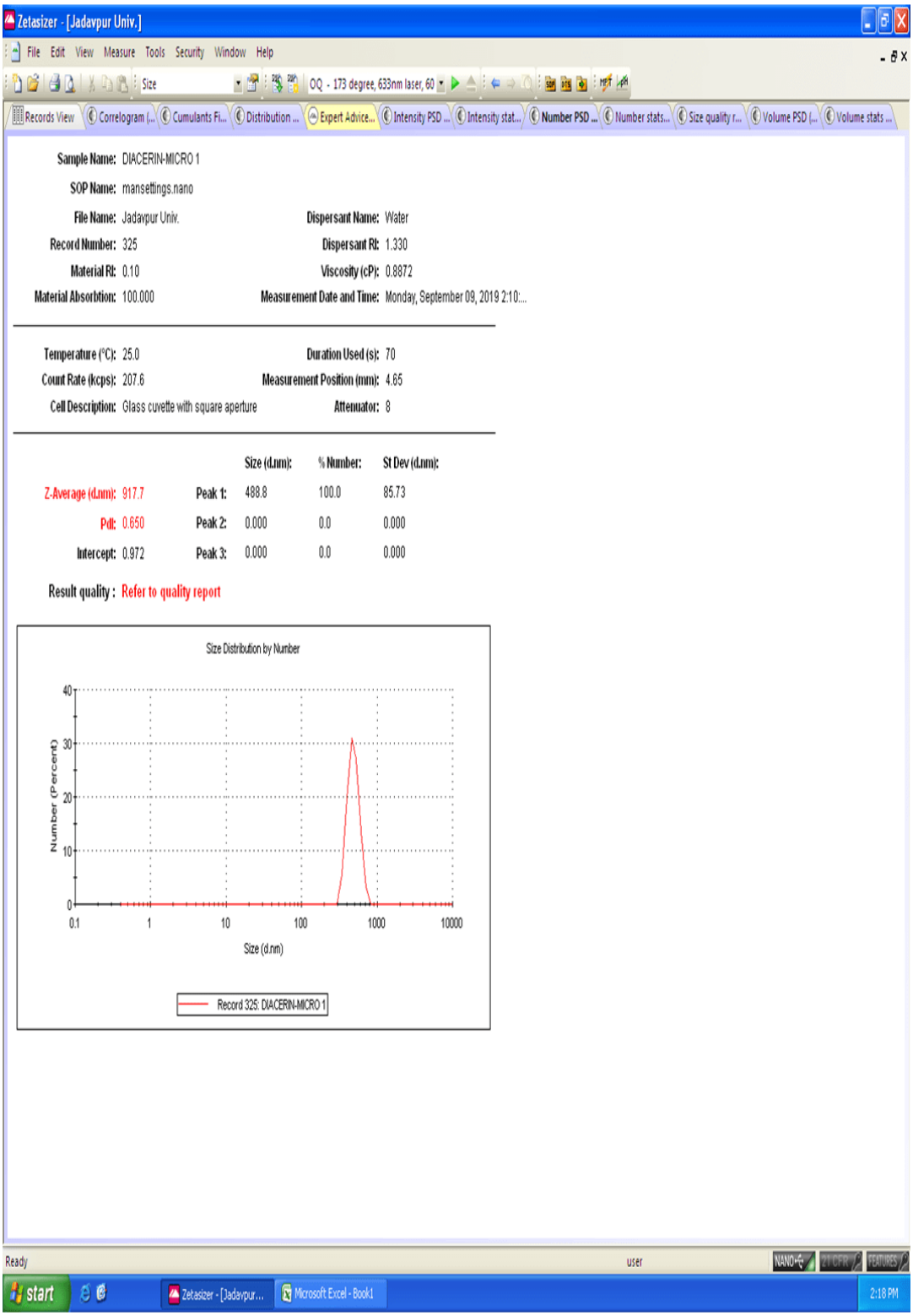

Zetasizer - [Jadavpur Univ.]
File Edit View Measure Tools Security Window Help
Size
OQ - 173 degree, 633nm laser, 60
Records View
Correlogram (...
Cumulants Fi...
Distribution ...
Expert Advice...
Intensity PSD ...
Intensity stat...
Number PSD ...
Number stats...
Size quality r...
Volume PSD (...
Volume stats ...
Sample Name: DIACERIN-MICRO 1
SOP Name: mansettings.nano
File Name: Jadavpur Univ.
Record Number: 325
Material RI: 0.10
Material Absorbtion: 100.000
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Measurement Date and Time: Monday, September 09, 2019 2:10:...
Temperature (°C): 25.0
Count Rate (kcps): 207.6
Cell Description: Glass cuvette with square aperture
Duration Used (s): 70
Measurement Position (mm): 4.65
Attenuator: 8
Size (d.nm): % Number: St Dev (d.nm):
Z-Average (d.nm): 917.7
Peak 1: 488.8 100.0 85.73
PdI: 0.650
Peak 2: 0.000 0.0 0.000
Intercept: 0.972
Peak 3: 0.000 0.0 0.000
Result quality : Refer to quality report
Size Distribution by Number
Number (Percent)
Size (d.nm)
Record 325: DIACERIN-MICRO 1
Ready
user
start
Zetasizer - [Jadavpur...
Microsoft Excel - Book1
2:18 PM

Figure 8.3

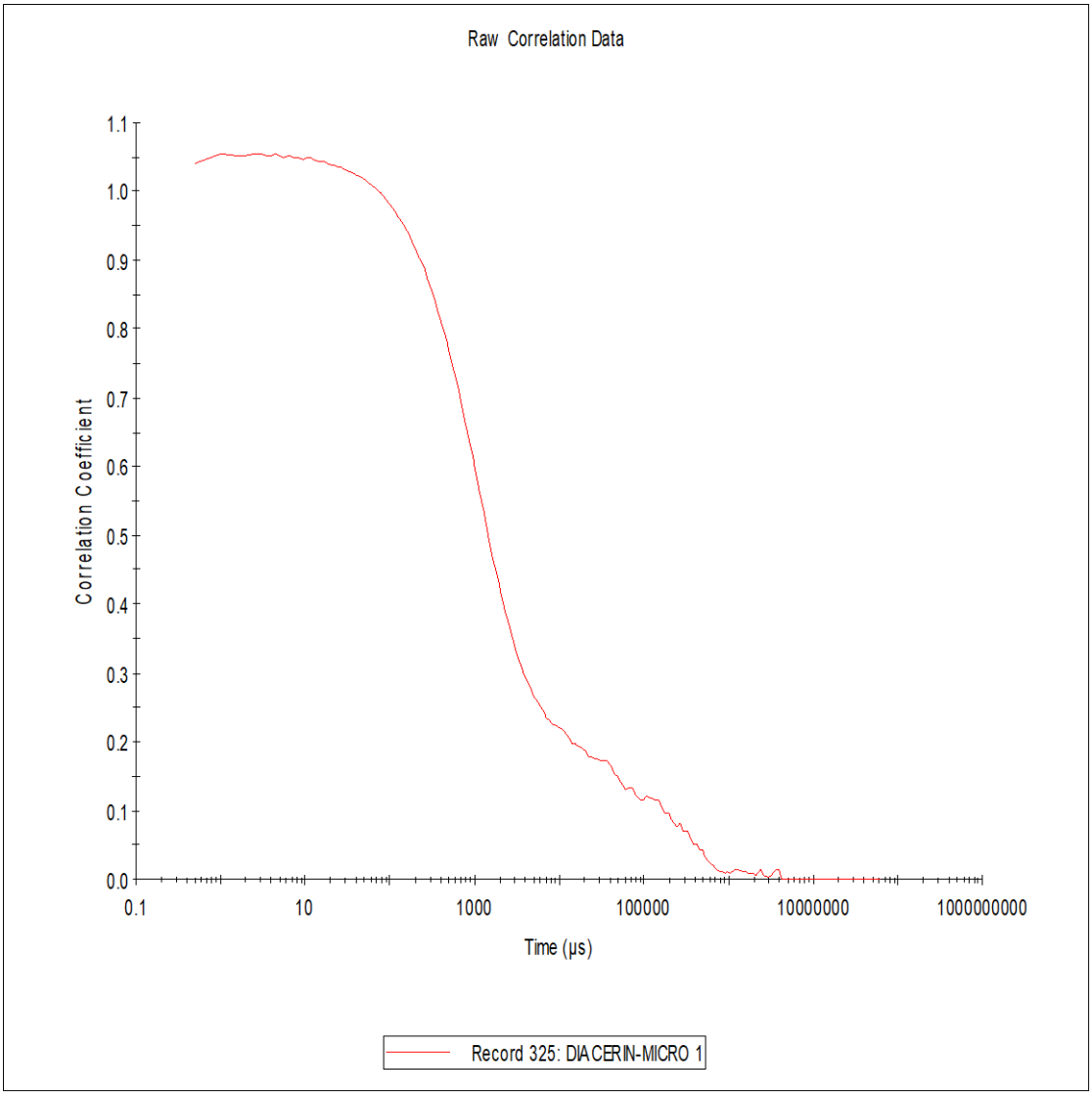


Figure 8.4

Malvern

Zeta Potential Report

v2.3

Malvern Instruments Ltd - © Copyright 2008

Sample Details

Sample Name: DIACERIN-MICRO 1

SOP Name: mansettings.nano

General Notes:

File Name: Jadavpur Univ.

Record Number: 326 , September 09, 2019 2:1...

Dispersant Name: Water

Viscosity (cP): 0.8872

Dispersant Dielectric Constant: 78.5

System

Temperature (°C): 25.0

Count Rate (kcps): 365.0

Cell Description: Zeta dip cell

Zeta Runs: 12

Measurement Position (mm): 4.50

Attenuator: 7

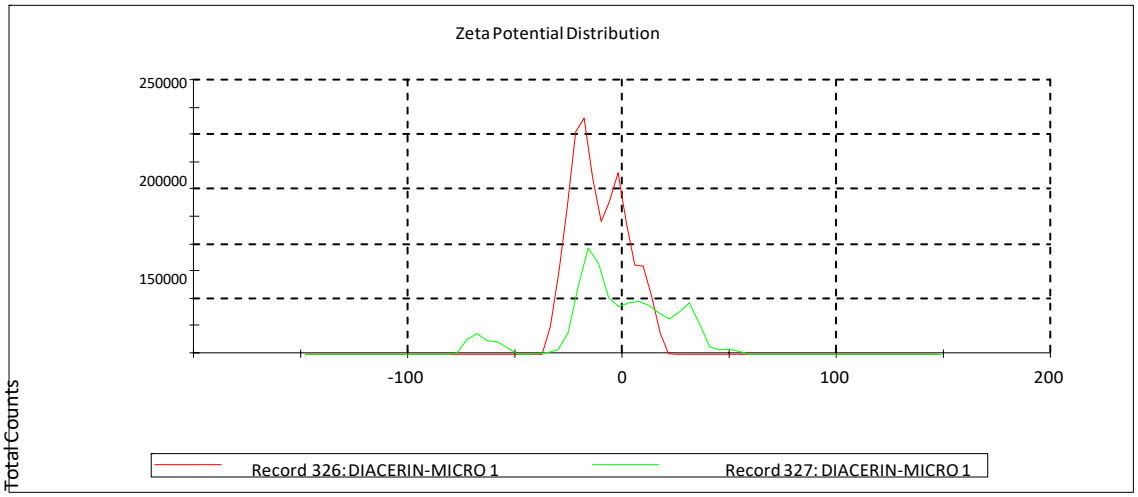
Results

		Mean (mV)	Area (%)	St Dev (mV)
Zeta potential (mV): -10.8	**Peak 1:**	-19.7	54.5	6.18
Zeta Deviation (mV): 12.4	**Peak 2:**	-0.0368	45.5	7.54
Conductivity (mS/cm): 0.0360	**Peak 3:**	0.00	0.0	0.00

Result quality: Good

Dispersant RI: 1.330

Date and Time: Monday



Malvern Instruments Ltd
www.malvern.com

Zetasizer Ver. 7.11
Serial Number: MAL1053771

File name: Jadavpur Univ.
Record Number: 326

09 Sep 2019 2:17:17 PM

Figure 8.5

CHAPTER-9

CHARACTERIZATION

E. FT-IR study to detect drug polymer and excipients interaction :

FT-IR spectroscopy using Shimadzu Japan (model Prestige 21) DCN, Polymer and excipients interaction was investigated. KBR dices were used for sample preparation (two mg sample taken for two hundred mg KBr). DCN (API), microsphere and drug, polymer, excipients physical mixture were taken for FT-IR spectroscopy in scanning region 4000- 400cm^{-1}(mid IR region)

Table 9.1: FTIR Spectrum and characteristic band of Diacerein pur drug (published literature)*, Diacerein API(used in the study), Diacerein microsphere, Drug excipients physical mixture.

Diacerein(pure drug)		Diacerein(API)	Microsphere	Physical mixture
C-H stretch aromatic	3068cm^{-1}	3067cm^{-1}	3329 cm^{-1}	3048cm^{-1}
C-H stretch Aliphatic Symmetric	2951cm-1	2835cm-1	2899cm-1	2867cm-1
C=O ester	1766cm-1	1763cm-1	1768cm-1	1763cm-1
C=O stretch COOH	1675cm-1	1676cm-1	1678cm-1	1620cm-1
C-O stretch Ester	1188cm-1	1253cm-1	1254 cm-1	1240cm-1
M-substituted Benzene	746cm-1	837cm-1	863 cm-1	878 cm-1
Benzene	700cm-1	760cm-1	761 cm-1	745 cm-1

* IJPSR, Jan 1 “Pre and post formulation comtibility study of Diacerein”, JDDT, 2019;9(4),454-460

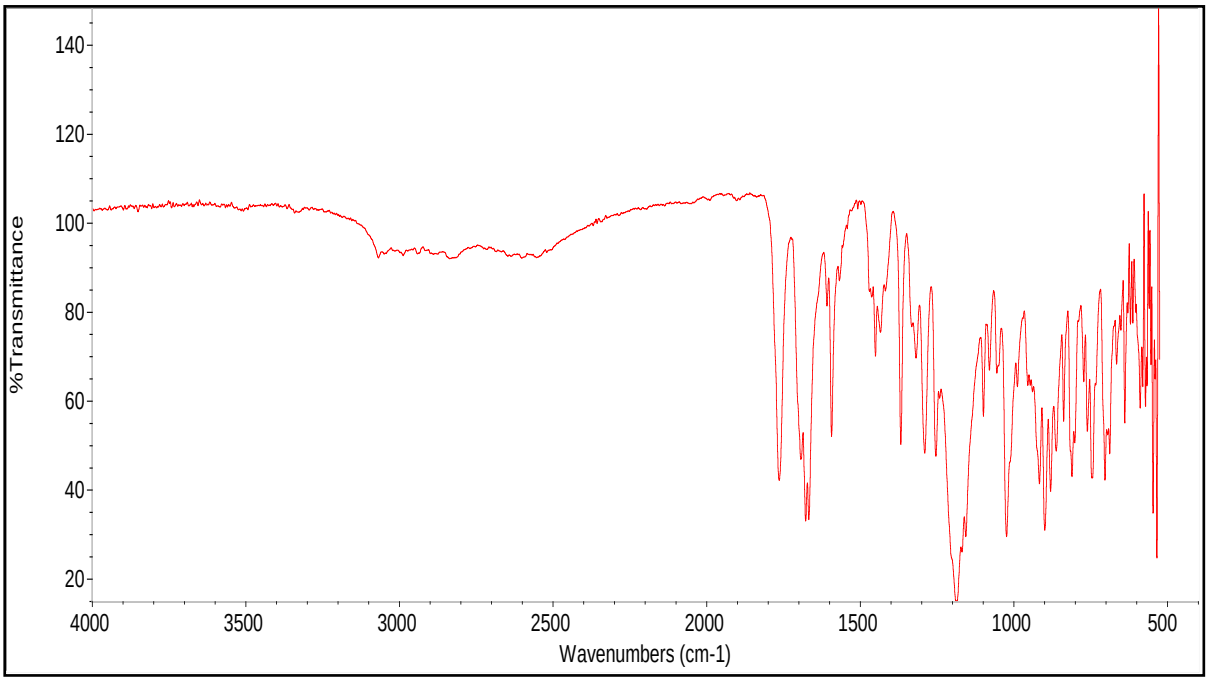


Figure 9.1: FTIR, of Diacerein Ph. Eur (API)

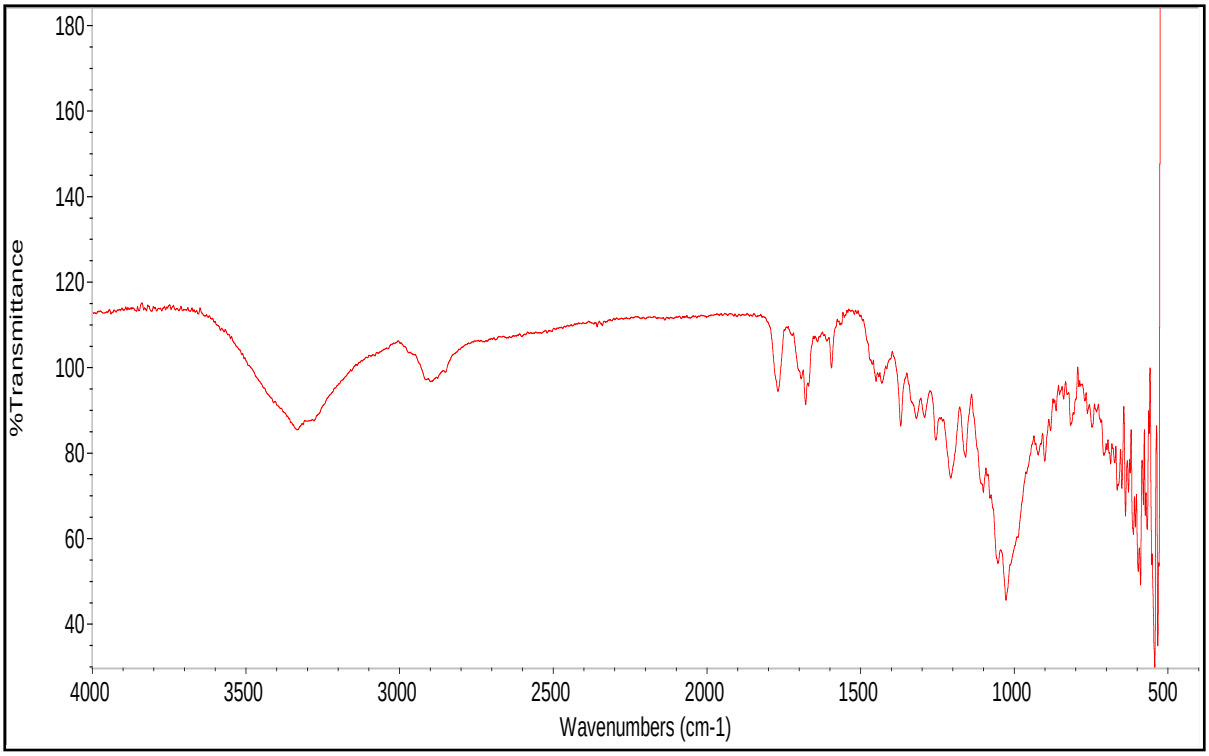


Figure 9.2: FTIR of Diacerein Microsphere

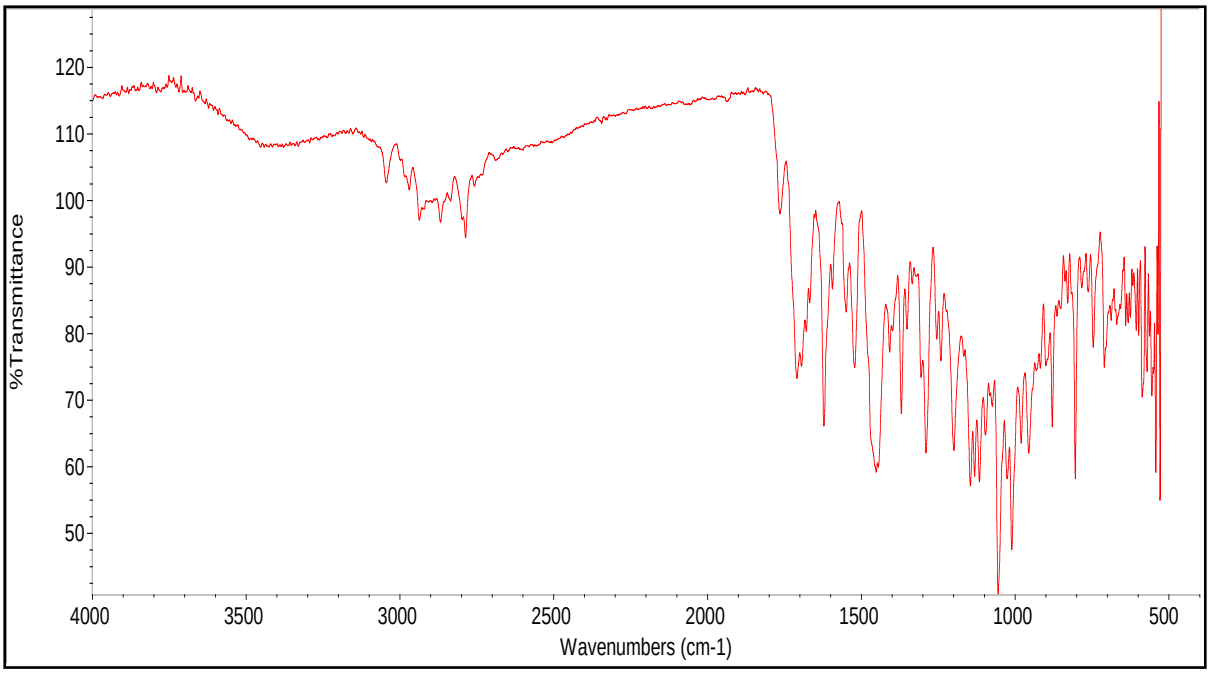


Figure.9.3: FTIR of Diacerein with other ingredients physical mixture.

Fourier Transform Infrared Spectroscopy (FT-IR Study) :

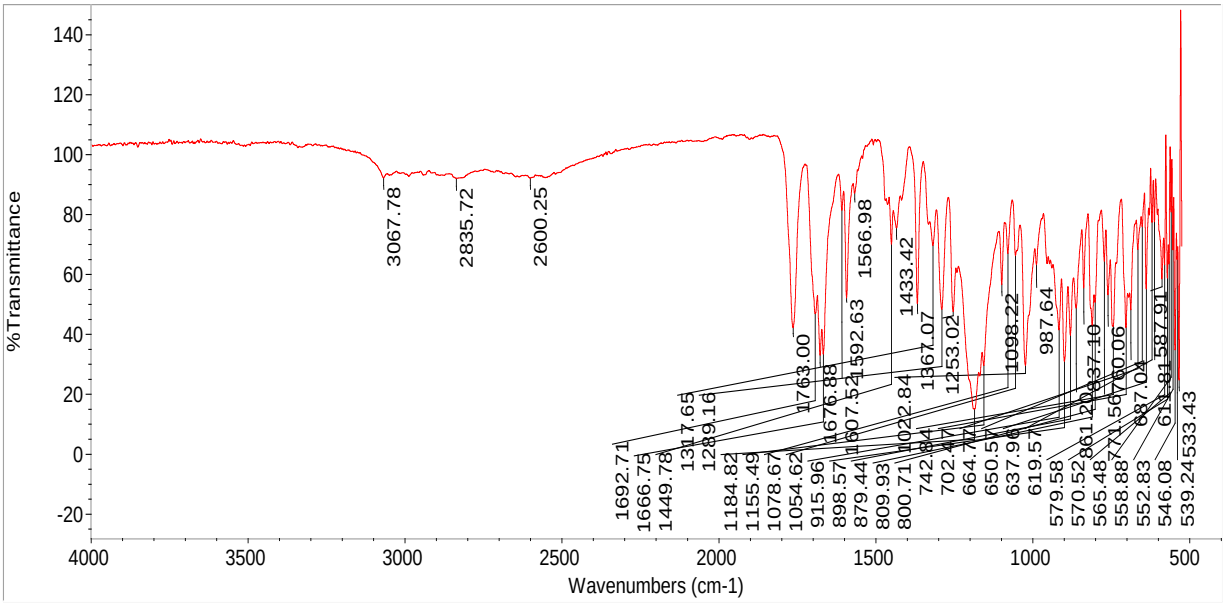


Figure 9.4: FTIR, of Diacerein API

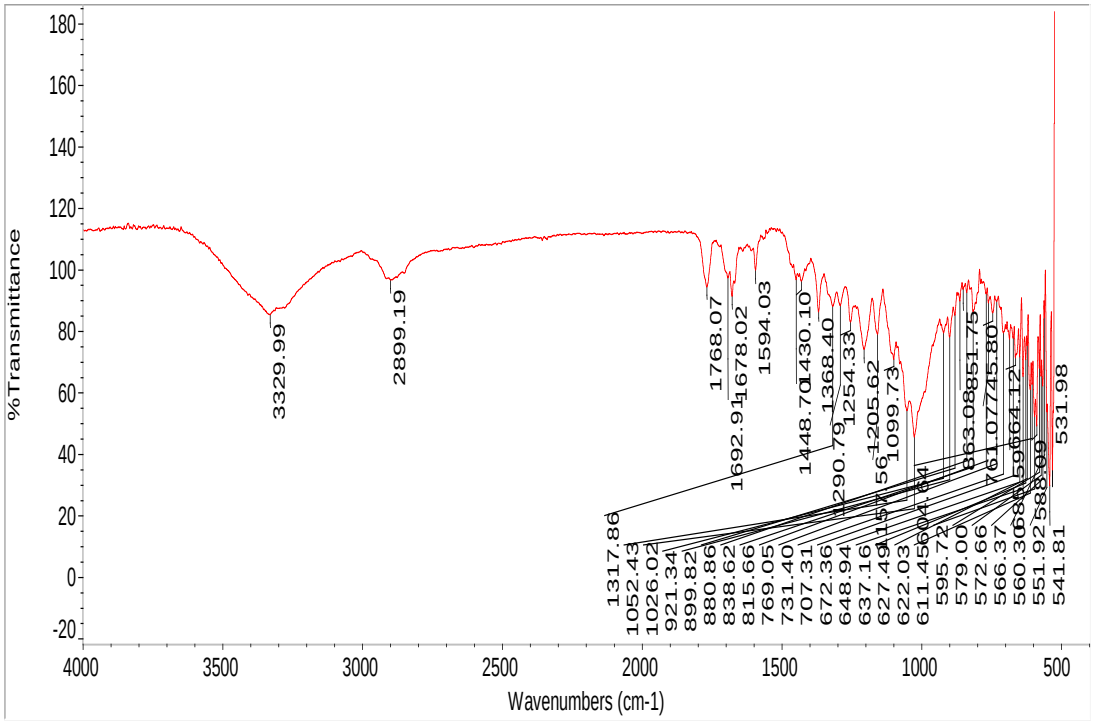


Figure 9.5: FTIR of Diacerein Microsphere

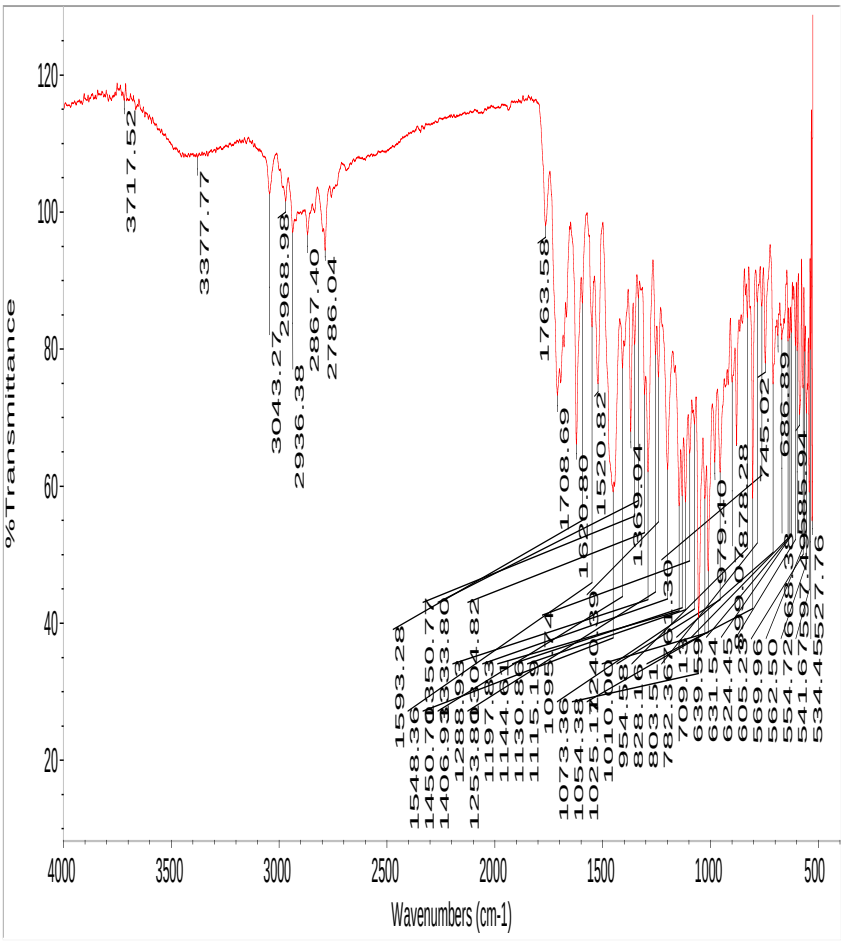


Figure 9.6: FTIR of Diacerein with other ingredients physical mixture.

F. X-RD Analysis for characterization of microsphers:-

X-ray diffraction analysis (XRD) is one of the micro structural analysis methods used for the identification of crystallimity of polymers, recognition of crystalline phases (polymorphism), and orientation of polymers.

It obeys the Braggs law and diffracted beams are often referred to as reflections. It is also used to determine crystal structure of drugs complexes.

$$L = d \sin\theta$$

$$n\lambda = 2d \sin\theta \text{ [d= lattice spacing].}$$

Where n = order of reflection, λ= wavelength of incident xrays,ɵ = angle of incidence
Instrument used : ULTIMA-III, RIGAKU MAKE (JAPAN), Cu target slit =10 mm.
Filter = (Cu) and Kb , voltage of 30 Kv and a current of 15 mA
Experiment temp := room temp.
scan speed and scan axis were 1.000 deg/min and 2θ/θ
respectively.

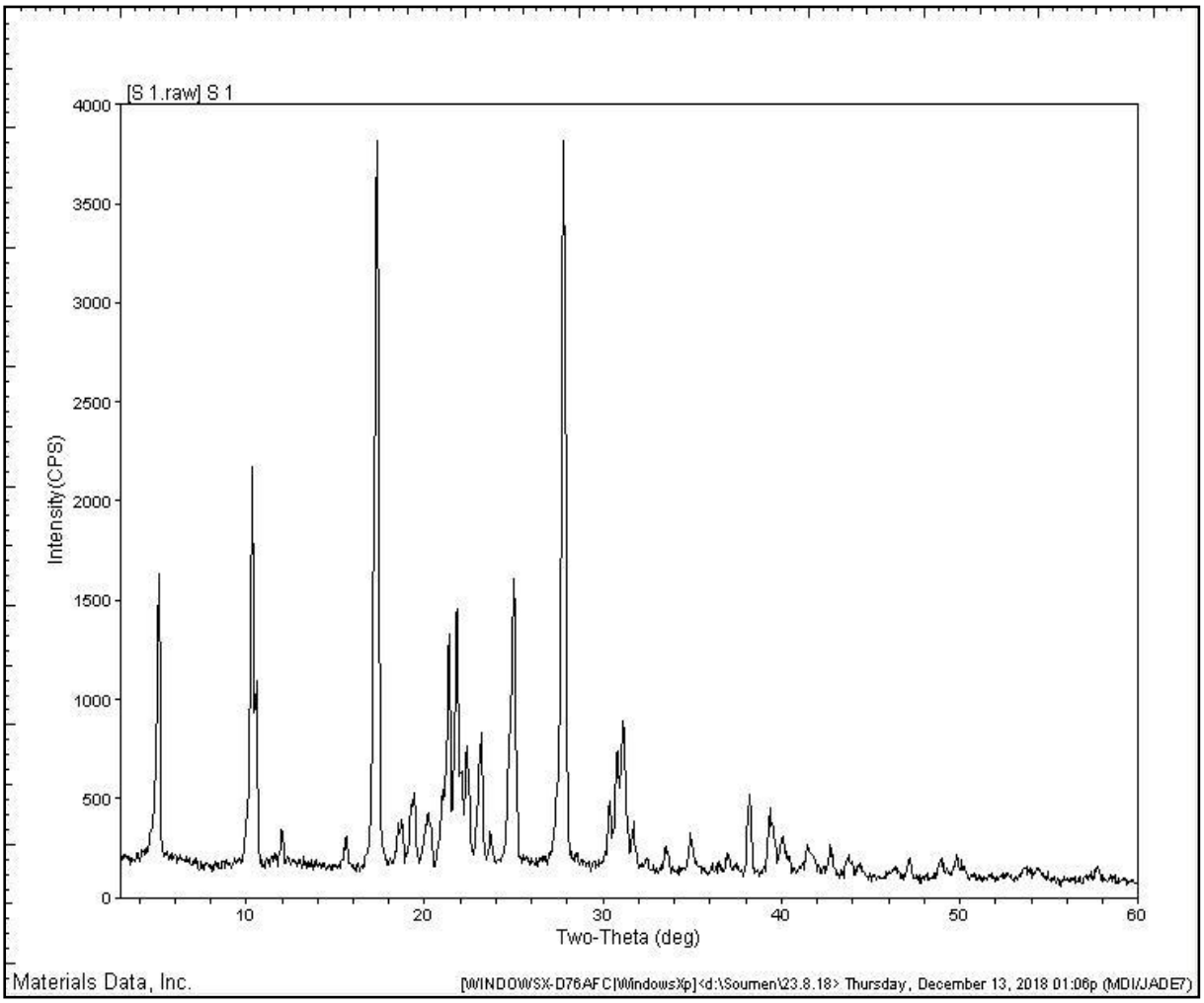


Figure 9.7: XRD of Diacerein API

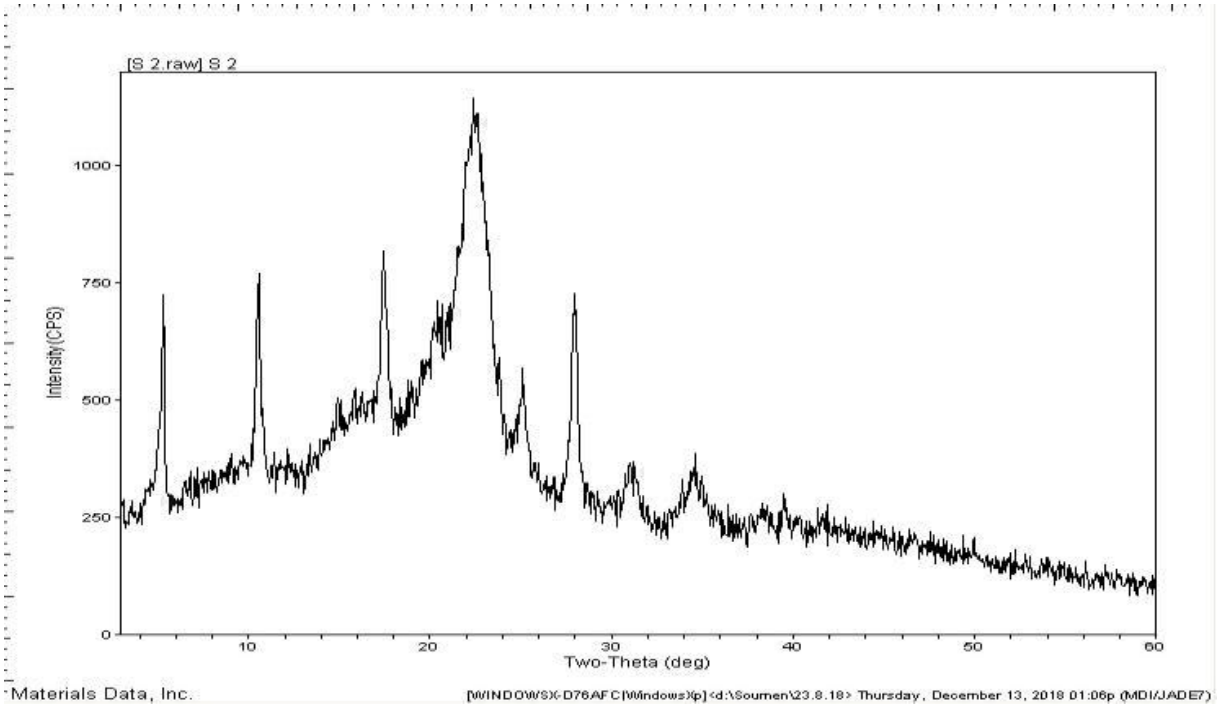


Figure 9.8: XRD of Diacerein Microsphere

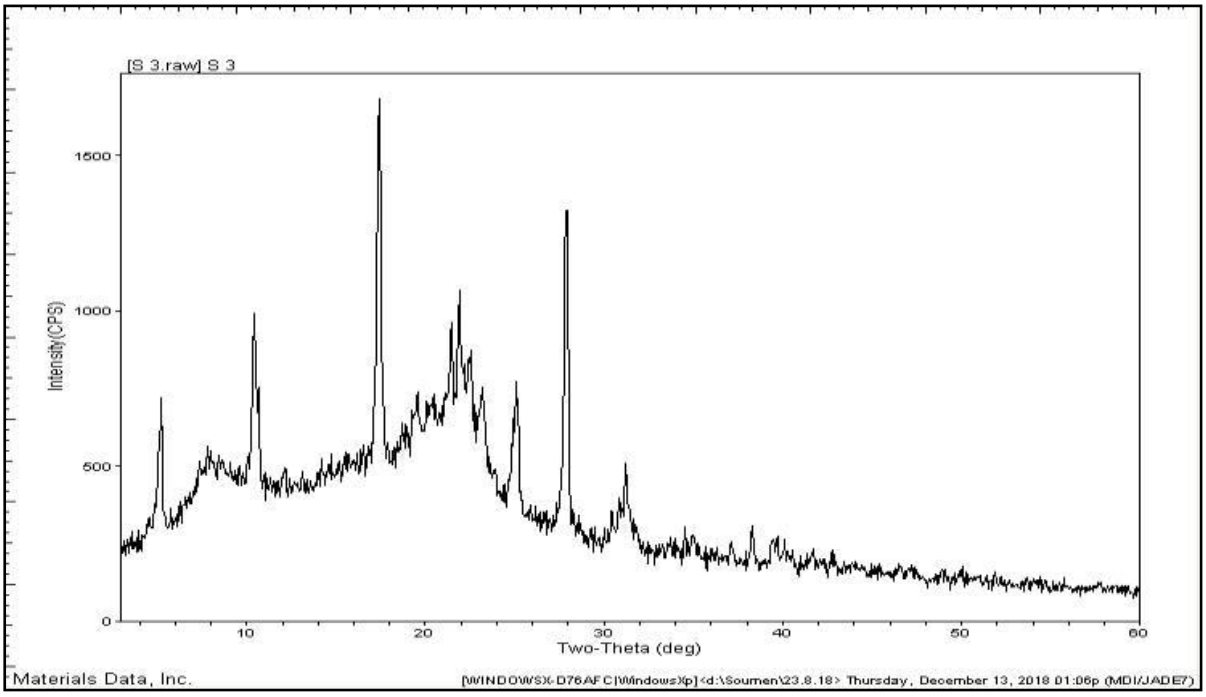


Figure 9.9: XRD of Physical Mixture of Diacerein and Ingredients

G. Differential Scanning Calorimetry:-

This study is a measurement of heat flow generated in a sample when the sample is heated or cooled or kept at constant temperature. DSC study can measure M.P, crystallization chemical

reaction etc. This study can deal with the influence of shape of DSC curve and can be used for quality study.

DSC study of DCN, microsphere and drug, polymer, excipients mixture was done as a characterization method in Perkin Elmer instrument (model DSC 4000), software pyres (heating range 50- 300^{0C}). DSC thermogram were shown in fig.24 to 26. Characteristic peak of DCN was changed in microsphere and physical mixture might be due to the presence of polymers and excipients. Change in peak temperature was within 10^{0C} .Which shows stability and compatibility of drug, polymer and excipients during the process.

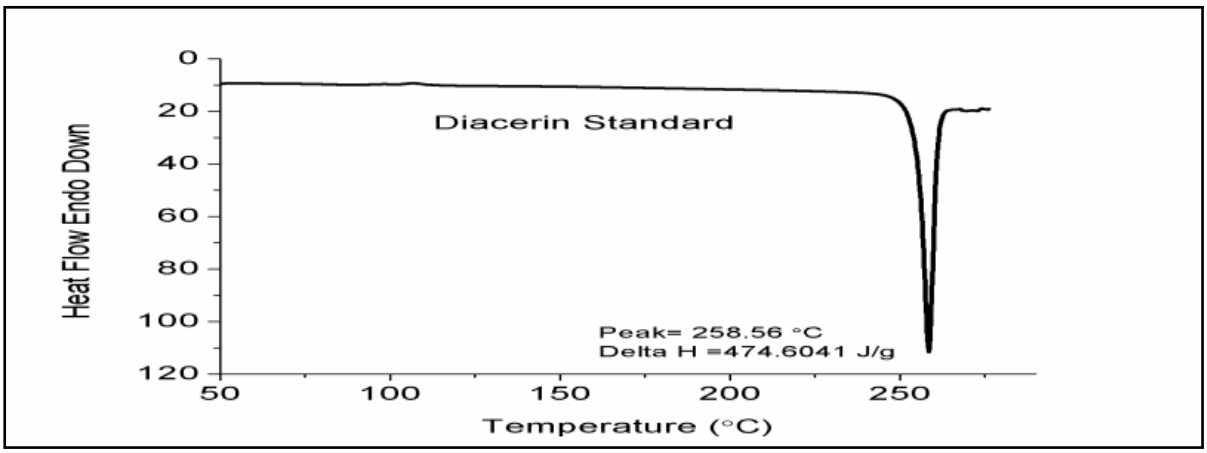


Figure 9.10: DSC curve of DCN

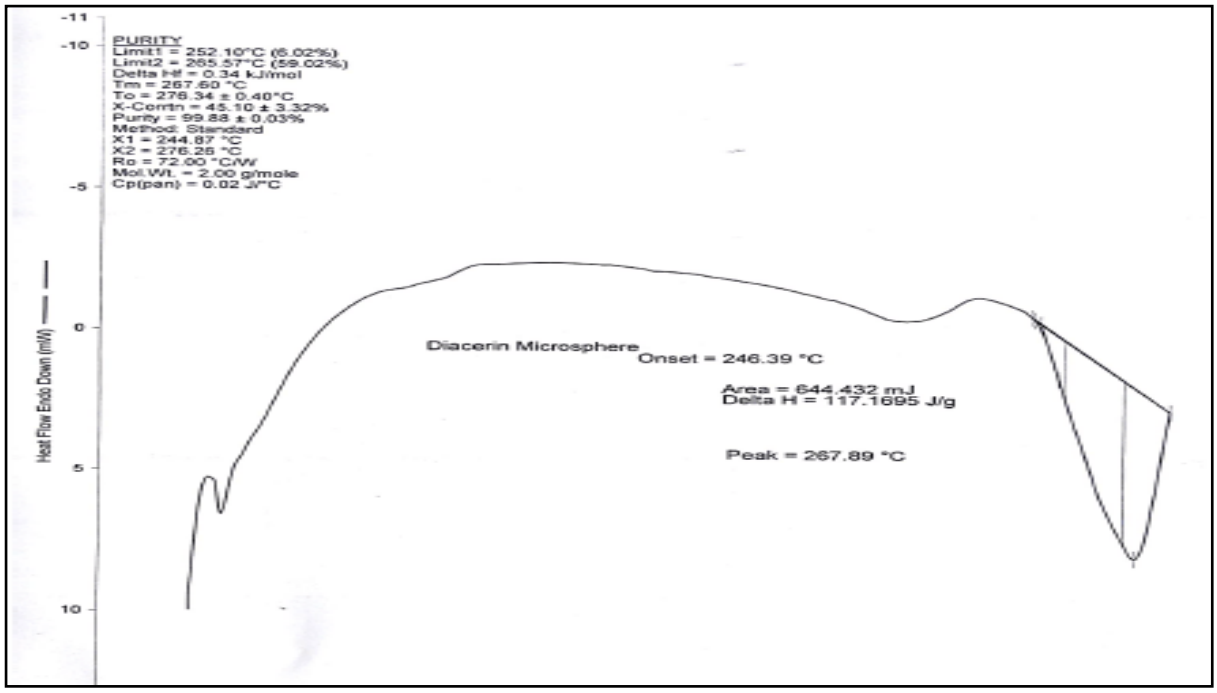


Figure 9.11: DSC study of Diacerein Microsphere

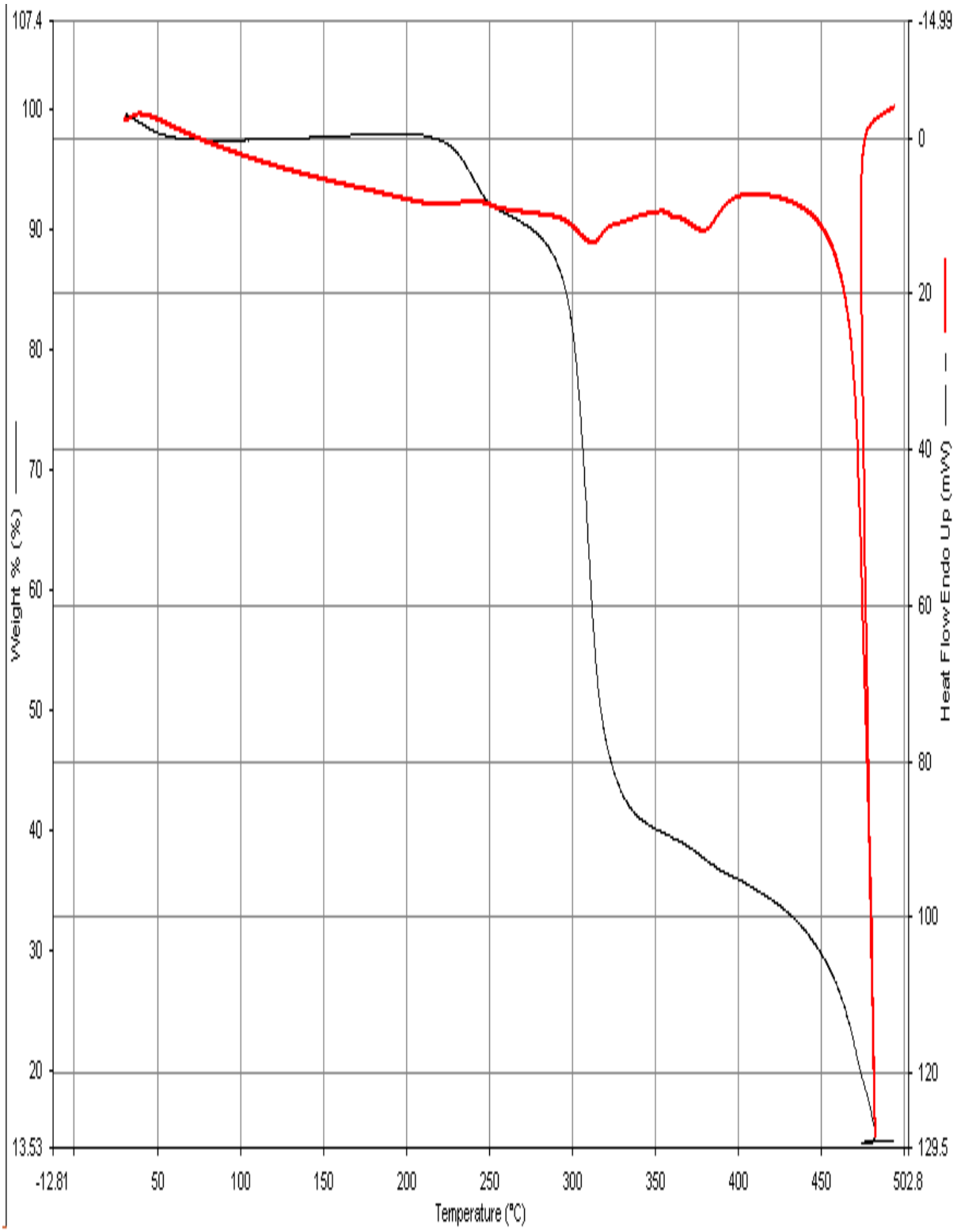


Figure 9.12: DSC curve of Physical Mixture of Diacerein, polymer and Ingredients

CHAPTER-10

DRUG RELEASE STUDY

H. Comparative In-Vitro dissolution rate study from Diacerein microsphere and comparison with conventional Diacerein capsule available in the market:-

Procedure:-

Dissolution can be defined as a process where solid substance solubilises in a given solvent. This can further be defined as transfer of mass from solid surface to liquid surface.

USP type-II dissolution test apparatus (paddle type) was used for in-vitro dissolution rate study for Diacerein microsphere and conventional capsule at temp 37^0C,speed 50 rpm and medium : phosphate buffer PH 6.8 and citrate buffer PH 6.0 and 0.1 N HCl. Quantity of dissolution medium (500ml). Weighed quanty of microsphere (eqv. to 50mg Diacerein) was added to the dissolution medium and at preset time interval of 1,2,4,8,12,16,20 and 24 hrs. 5 ml of aliquot was withdrawn in each time and replaced by an equal volume of fresh dissolution medium. Aliquots following suitable dilution were analyzed by UV visible spectrophotometer at the wavelength of 254 nm for phosphate buffer and 0.1N Hcl medium and at 340 nm for citrate buffer (ref. IP 2014, PP. 1543-1545). In the same manner the dissolution rate study was repeated for conventional Diacerein capsule 50mg available in the market. The study showed sustained release action of microsphere in vitro.

Dissolution rate of Diacerein microsphere (Summary of six capsules) :-

Table: 10.1

Time (hr)	Av.% release in Phosphate buffer pH 6.8	Av.% release in citrate buffer pH 6.0	Av.% release in 0.1 N HCl
1 hr	10.62	8.28	2.92
2 hr	22.42	20.01	3.24
4 hr	42.38	31.89	3.86
8 hr	63.75	38.58	3.52
12 hr	70.40	48.03	2.63
16 hr	89.27	50.12	2.13
20 hr	97.60	56.48	2.12
24 hr	73.3	57.65	2.08

Table: 10.2

Diacerein 50mg conventional capsule (marketed preparation) :-

Cumulative % release (6 Capsules)

Time(hr)	Citrate buffer pH 6.0	Phosphate buffer pH 6.8	Acidic medium 0.1N Hcl
30 min	58.49	73.12	5.65
1 hr	82.95	80.39	6.16
2 hr	83.15	82.11	6.55
3 hr	80.42	81.21	6.70
4 hr	81.88	81.56	7.49

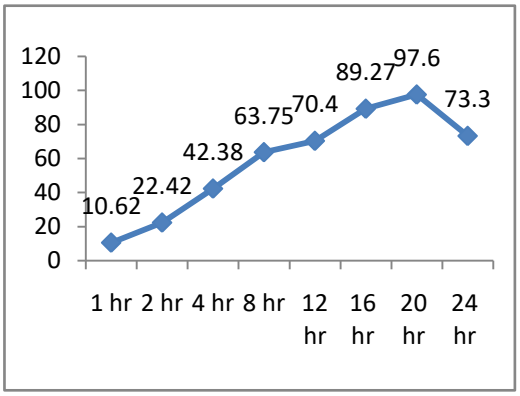
Table: 10.3
Diacerein microsphere release kinetics data:

Model Name	R^2	Slope	Intercept
Zero order model	R^2=0.985	3.745	15.09
First order model	R^2=0.847	0.235	2.067
Higuchi model	R^2=0.988	24.71	11.06
Korsmeyer-pappas model	R^2=0.971	0.713	1.106
Hixson Crowel	R^2=0.977	0.0153	4.599

Regression co-efficient R^2=0.988 Higuchi model in the best fit release kinetics model for Diacerein microsphere.

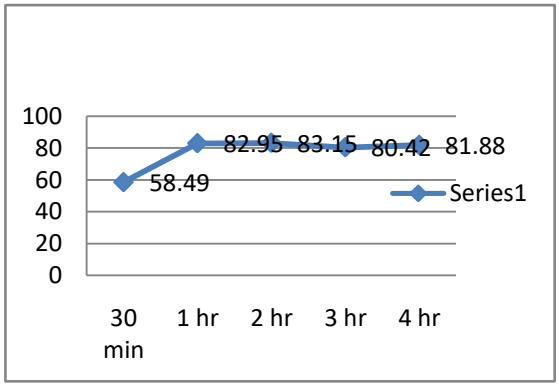
In vitro Drugs release from Diacerein Microsphere & Diacerein Capsule (market prapn.) at different buffered medium. (Plot of average % drug release vs time.hr):-

Figure 10.1



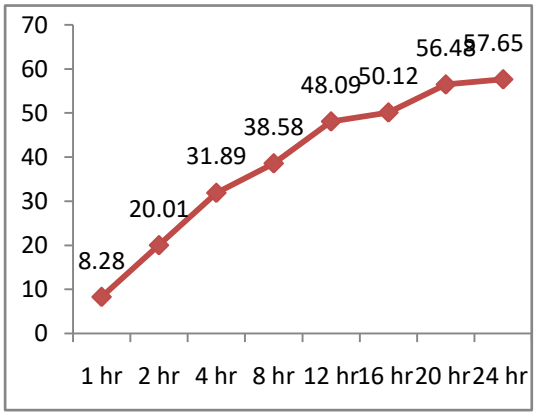
Diacerein microsphere in phosphate buffer pH 6.8

Figure 10.2



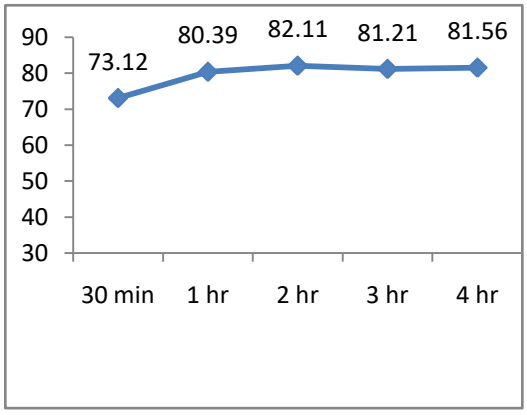
Diacerein capsule in Citrate buffer pH 6.0

Figure 10.3



Release of Diacerein microsphere in Citrate Buffer pH 6.0

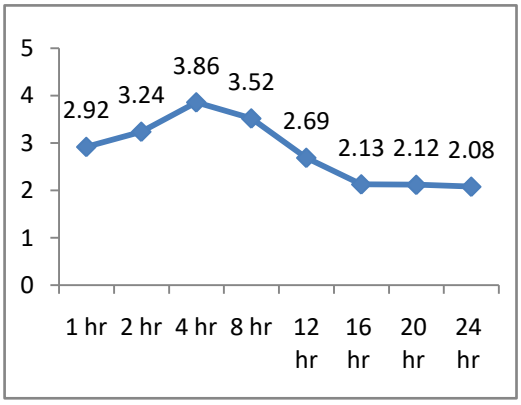
Figure 10.4



Release se of Diacerein capsule (M.P) in Release of DCN capsule in phosphate buffer ph 6.8

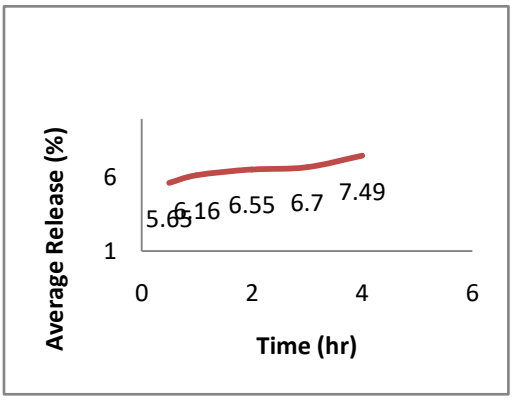
Release of DCN microsphereat 0.1N Hcl

Figure 10.5



Release of DCN capsule in 0.1N Hcl

Figure 10.6



Release Kinetics of Diacerein of Microsphere in phosphate buffer pH 6.8

Figure 10.7

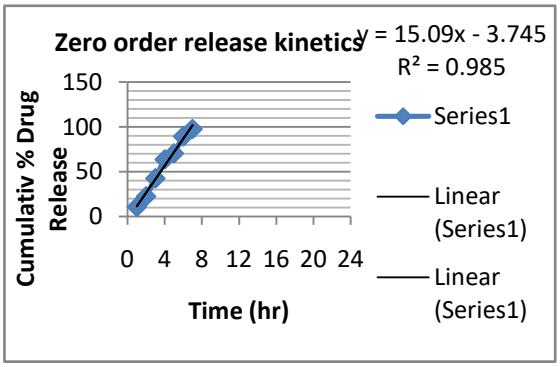


Figure 10.8

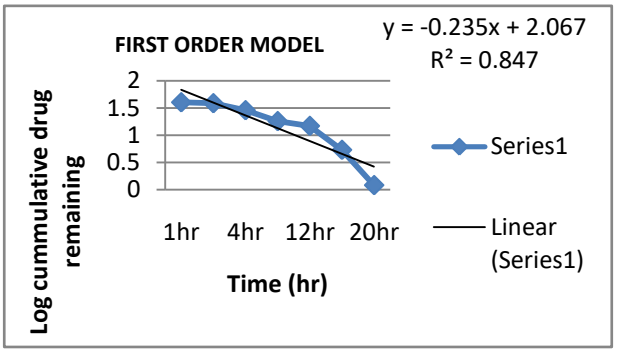


Figure 10.9

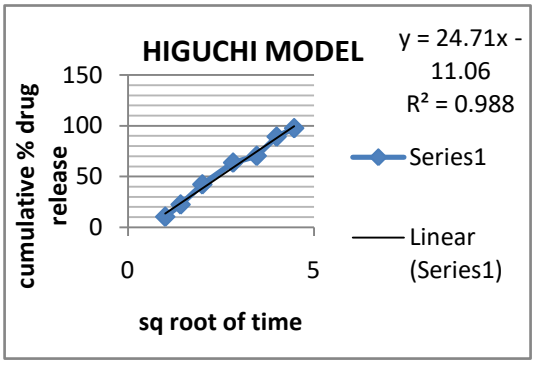


Figure 10.10

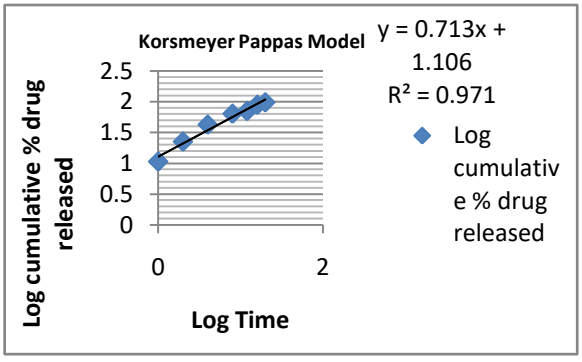
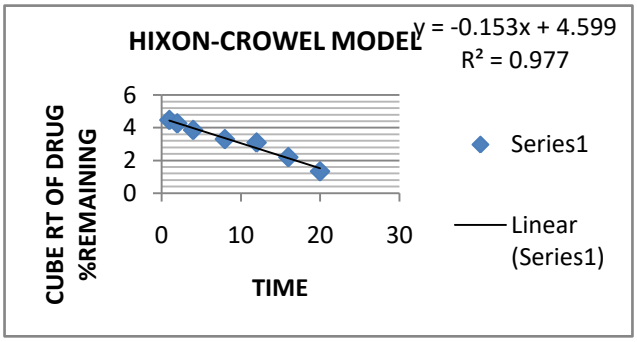


Figure 10.11



CHAPTER-11

PHARMACOKINETIC STUDY OF MICROSPHERE AND COMPARISON WITH MARKETED PREPARATION

Analytical Method Development and Evaluation of Diacerein microsphere formulation by HPLC

INTRODUCTION:

A method has been developed for validation assay of **Diacerein** in Rabbits plasma using **Aspirin** as internal standard (IS).

OBJECTIVES:

To describe the development of proposed method of analysis of **Diacerein** from Rabbits plasma.

CHEMICALS:

Methanol – HPLC Grade
Potassium Dihydrogen Phosphate – HPLC Grade
Acetonitrile – HPLC Grade
Phosphoric acid – AR Grade
Perchloric acid – AR Grade

CHROMATOGRAPHIC PARAMETERS:

Component Name	HPLC
Component ID	Binary gradient
Manufacturer	Shimadzu Corp.
Model	SPD-M20A 230V
Serial Number	L20154705630
Column	Thermo 250-4,6 5µm C18
Mobile Phase	Phosphate buffer (pH 3) : ACN (55:45)
Flow rate	0.8ml/min.
Temparature	Ambient

Shimadzu HPLC Properties

Shimadzu HPLC system Injection Volume = 20.00 ul
Shimadzu LC Method Parameters
Pumps
=====
Pump A Model: LC-20AD
Pump B Model: LC-20AD
Pumping Mode: Binary Flow
Total Flow: 0.8000 mL/min.
Pump B Pct: 55.0 %
B Curve: 0
Pressure Range (Pump A/B): 0.0 – 6000 psi

Internal Standard Concentration:

Aspirin = 500mcg/ml

STUDY DESIGN:

In- vivo bioavailability studies were performed in DCN microsphere and DCN capsule (marketed Preparation) comparatively to study their release pattern. DCN microsphere showed sustained/ delayed release action in in-vitro dissolution rate study. Hence, it was required to analyse in- vivo release pattern. The study protocol was approved by Animal Ethics Committee of JU. Six healthy albino rabbit weighing 1.5- 2.5 kg were obtained from registered animal breeder and were acclimatized for ten days. Each animal was fur marked and allocated separate cage. Animals were kept in fasting condition for 12 hr and water was added adlibidum. Fasting blood samples were withdrawn early morning form marginal ear vein. Both DCN microsphere and DCN capsule were administered at dose of 10mg/kg body weight through feeding tube/esophageal tube. Blood samples were withdrawn in a quantity of 0.5 ml and at predetermined time gap of 0.5,1.0,1.5,2,2.5,3,4,6,8,12,18 and 24hrs using disposal syringe.

RIA vials were utilized for collection of blood using heparin as anticoagulant. Blood samples were centrifuged at 4000rpm for 5min for separation of plasma.

PLASMA EXTRACTION PROCEDURE:

150µl rabbit plasma, and 50µl of IS (Internal standard Aspirin) were added to 1.5 ml polypropylene micro-centrifuge tubes. Deproteinization of the samples were done by addition of 300µl of 10% Perchloric acid. Mixing was done by vortex mixture . Thereafter, centrifuged at 12000 rpm for 5min. Accurately taken 20µm of the upper clean layer was injected directly into the chromatographic system.(10)

PLASMA CALIBRATION CONCENTRATIONS (mcg/ml):

0.37, 0.5, 1, 2, 4, 8, 10, 12 µg/ml.

SENSITIVITY

QC Point	Concentration
LLOQ	0.37 mcg/ml
LQC	1.11 mcg/ml
MQC	6 mcg/ml
HQC	15 mcg/ml

CALIBRATION CONCENTRATION (mcg/ml)

Table 11.1: HPLC Data Table

	0.37	**0.5**	**1**	**2**	**4**	**8**	**10**	**12**
Lin 1	0.37763	0.487496	0.936038	1.880342	3.880472	7.904943	9.925463	12.00421
Lin 2	0.380731	0.485105	0.974567	1.945479	3.85779	7.770797	9.928327	12.1049
Lin 3	0.381022	0.501888	0.906409	1.878439	3.883094	7.890105	9.927175	12.00433
mean	0.380	0.491	0.939	1.901	3.874	7.855	9.927	12.038
s.d.	0.002	0.009	0.034	0.038	0.014	0.074	0.001	0.058
c.v.	0.495	1.847	3.640	2.007	0.359	0.936	0.015	0.483
nominal	102.647	98.299	93.900	95.071	96.845	98.191	99.270	100.315

Table 11.2: HPLC Data table

	Slope(M)	**Intersept-C**	**R square**
Lin 1	0.055944	-0.00314	0.999935
Lin 2	0.05636	-0.00282	0.999765
Lin 3	0.055805	-0.00329	0.999921
MEAN	0.056036	-0.00308	0.99987
S.D.	0.000289	0.00024	0.000094
C.V.%	0.5154	-7.7860	0.00944

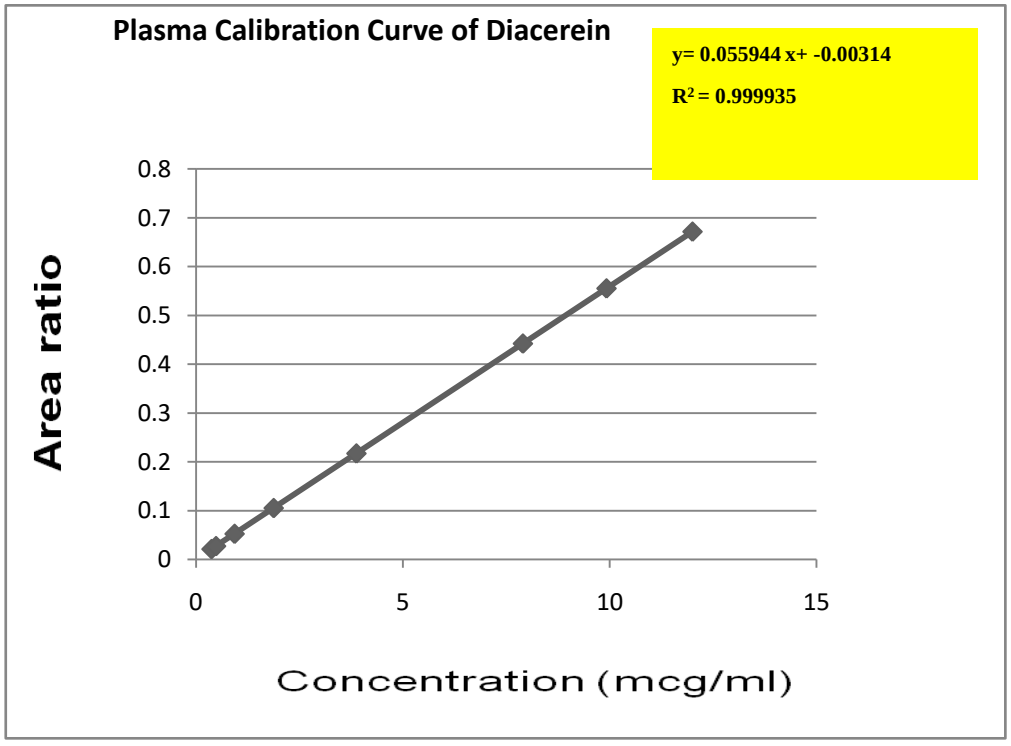


Figure 11.1

Table 11.3: HPLC DATA TABLE

Recovery :-

Inj.	Inj. In Dilluent			Inj in Plasma		
	1.11mcg/ml	**6mcg/ml**	**15mcg/ml**	**1.11mcg/ml**	**6mcg/ml**	**15mcg/ml**
1	113.084%	117.138%	115.644%	115.867%	113.260%	114.229%
2	107.886%	115.162%	115.072%	117.221%	113.295%	116.354%
3	115.386%	105.830%	113.072%	111.477%	110.414%	112.523%
Mean %	112.119%	112.710%	114.596%	114.855%	112.323%	114.369%
	% Recovery			102.441%	99.657%	99.802%

Table 11.4: HPLC DATA TABLE

Inter Day Precision Accuracy:

	1.11mcg/ml	**6mcg/ml**	**15mcg/ml**
Day 1%	101.072	104.350	114.229
Day 1%	101.354	101.353	116.354
Day 1%	89.932	104.613	112.523
Day2%	88.262	102.386	106.531
Day2%	85.029	105.115	107.518
Day2%	82.439	101.974	108.657
Day 3%	87.974	104.329	106.652
Day 3%	89.977	104.759	106.630
Day 3%	99.649	104.792	108.212
Mean %	91.743	103.741	109.701
S.D	7.126	1.422	3.701
C.V. %	7.768	1.370	3.374
Absolute percent bias (%)	8265.145	1729.020	731.338

Table 11.5 HPLC DATA TABLE

Intra Day Precision Accuracy

	1.11 mcg/ml	**6 mcg/ml**	**15 mcg/ml**
1	101.072%	104.350%	106.531%
2	101.354%	101.353%	107.518%
3	89.932%	104.613%	108.657%
Mean%	97.453%	103.439%	107.569%
SD	6.515	1.811	1.064
CV	6.685	1.751	0.989
Absolute percent basis (%)	8779.520	1723.978	717.124

Table 11.6: HPLC DATA TABLE

STABILITY

Freshly Thaw			Freeze Thaw		
1.11 mcg/ml	**6 mcg/ml**	**15 mcg/ml**	**1.11 mcg/ml**	**6 mcg/ml**	**15 mcg/ml**
101.072%	**104.350%**	**106.531%**	**88.262%**	**102.386%**	**106.652%**
101.354%	**101.353%**	**107.518%**	**85.029%**	**105.115%**	**106.630%**
89.932%	**104.613%**	**108.657%**	**82.439%**	**101.974%**	**108.212%**
97.453%	**103.439%**	**107.569%**	**85.243%**	**103.158%**	**107.165%**
% Stability			**114.323**	**100.272**	**100.377**

Table-11.7: Plasma concentration of Diacerein Capsules in Rabbit (1 – 6) Plasma

Time	Rabbit-1	Rabbit - 2	Rabbit-3	Rabbit - 4	Rabbit-5	Rabbit - 6	Mean
0.0	0	0	0	0	0	0	0
0.5	0.60	0.75	0.64	0.77	0.72	0.74	0.70
1.0	1.32	1.45	1.27	1.19	1.55	1.50	1.38
1.5	1.76	2.05	1.94	1.76	2.11	2.25	1.98
2.0	2.91	3.19	3.24	3.06	3.43	3.51	3.22
2.5	3.97	**4.05**	**3.86**	**3.77**	3.91	**4.11**	3.95
3.0	**4.02**	3.94	3.57	3.34	**4.00**	3.72	3.77
3.5	3.94	3.55	3.28	3.10	3.84	3.51	3.54
4.0	2.35	2.98	2.65	2.57	2.51	2.74	2.63
5.0	2.08	2.15	2.20	1.98	1.95	1.97	2.06
6.0	1.89	1.37	1.29	1.45	1.36	1.26	1.44
10.0	0.25	0.45	0.42	0.63	0.47	0.53	0.46
24.0	0	0	0	0	0	0	0
C max (mcg/ml)	**4.02**	**4.05**	**3.86**	**3.77**	**4.00**	**4.11**	**3.97**
T max (hrs.)	**3.0**	**2.5**	**2.5**	**2.5**	**3.0**	**2.5**	**2.67**
Auc 0-t (mcg-hr/ml)	**18.33**	**18.20**	**17.15**	**17.29**	**17.95**	**17.91**	**17.80**
Auc 0-∞ (mch-hr/ml)	**18.97**	**19.66**	**18.52**	**19.88**	**19.48**	**19.76**	**19.38**
Kel(hr-1)	**0.392**	**0.308**	**0.306**	**0.243**	**0.309**	**0.286**	**0.31**
T 1/2 (hrs.)	**1.77**	**2.25**	**2.26**	**2.85**	**2.25**	**2.43**	**2.30**

Time Interval	Mean Plasma Conc.
0.0	0
0.5	0.70
1.0	1.38
1.5	1.98
2.0	3.22
2.5	3.95
3.0	3.77
3.5	3.54
4.0	2.63
5.0	2.06
6.0	1.44
10.0	0.46
24.0	0

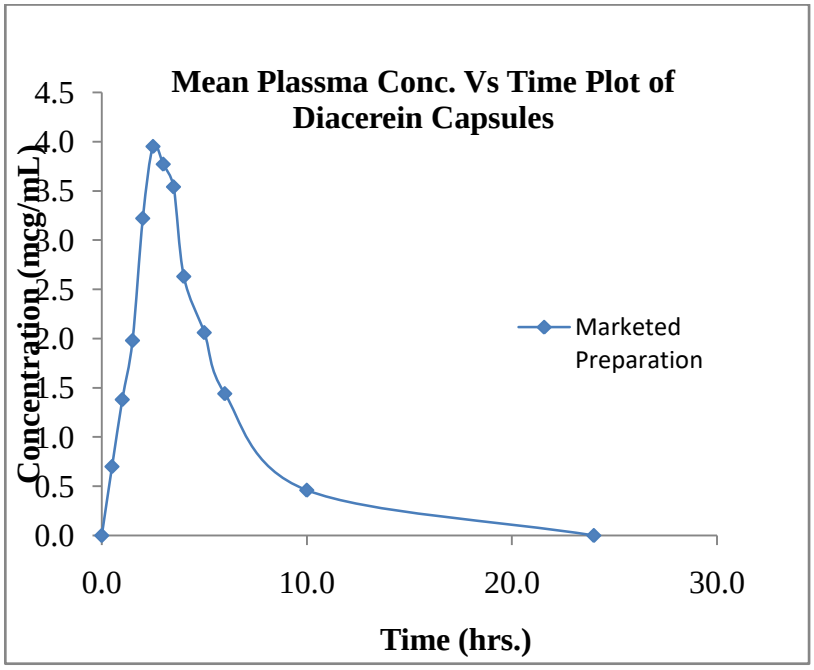


Figure 11.2

Figure: 11.3 **Graphical representation of plasma concentration of Diacerein capsule in rabbit (1-6)**

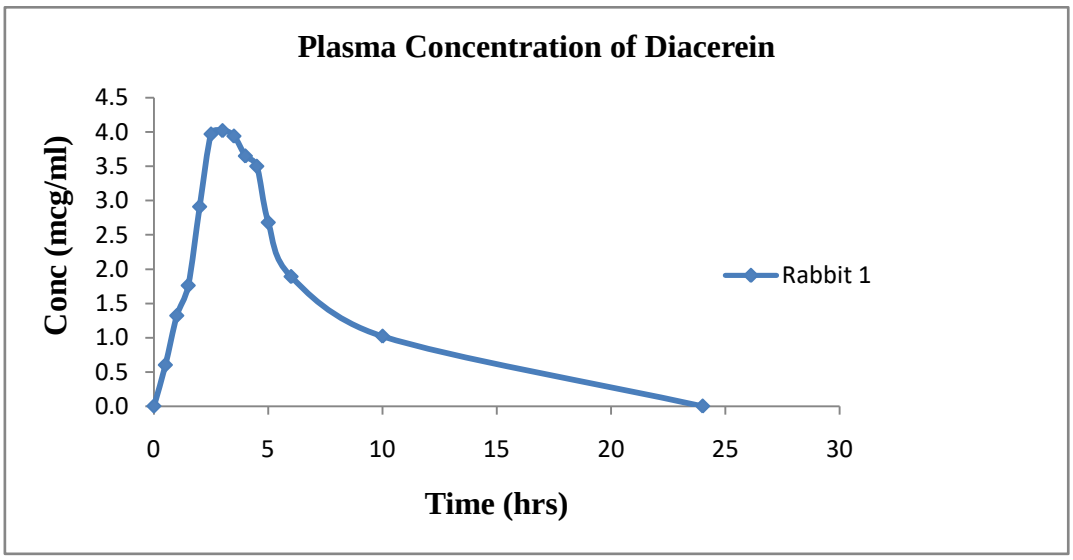


Figure 11.3

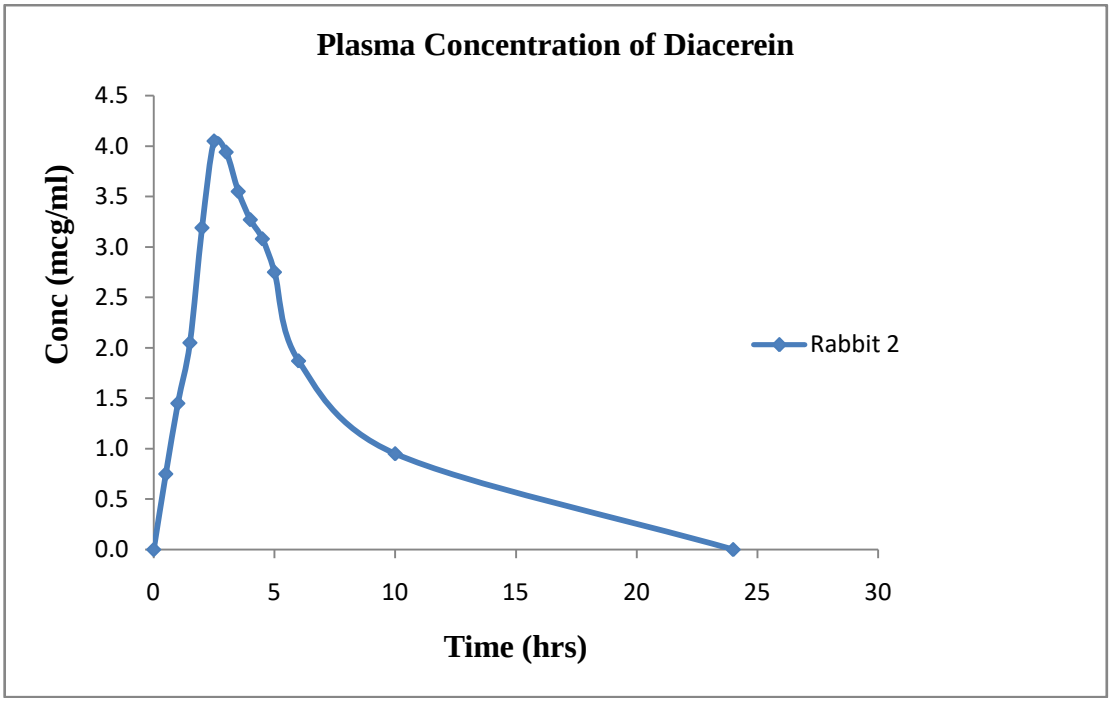


Figure 11.4

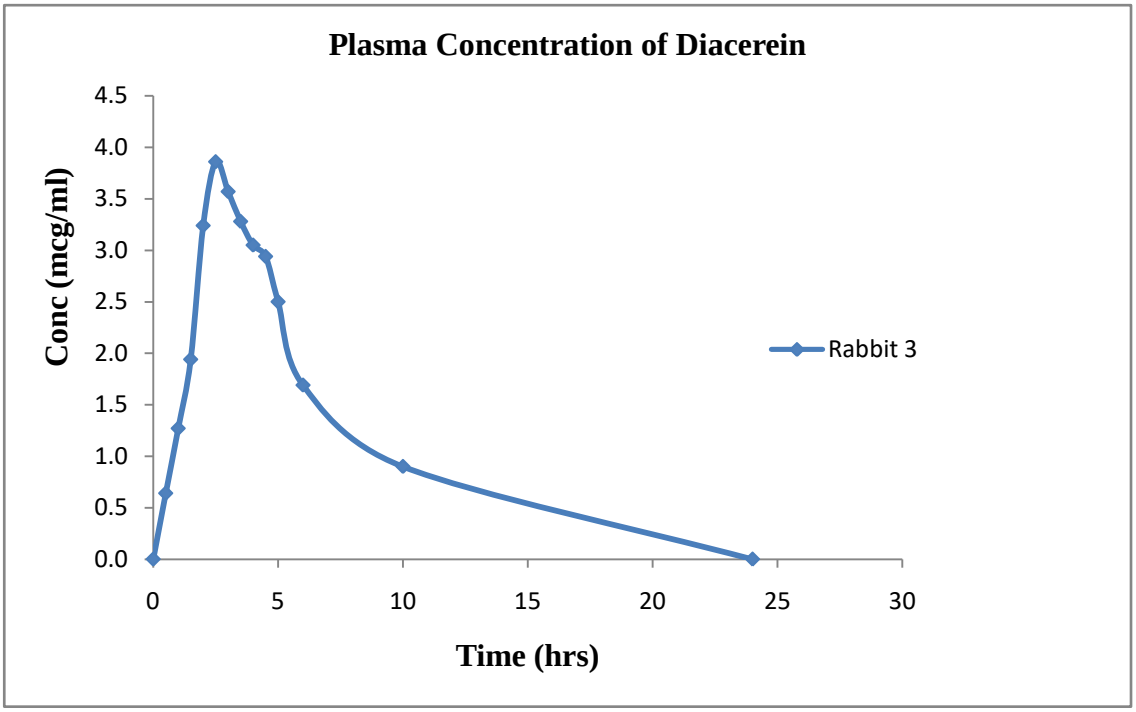


Figure 11.5

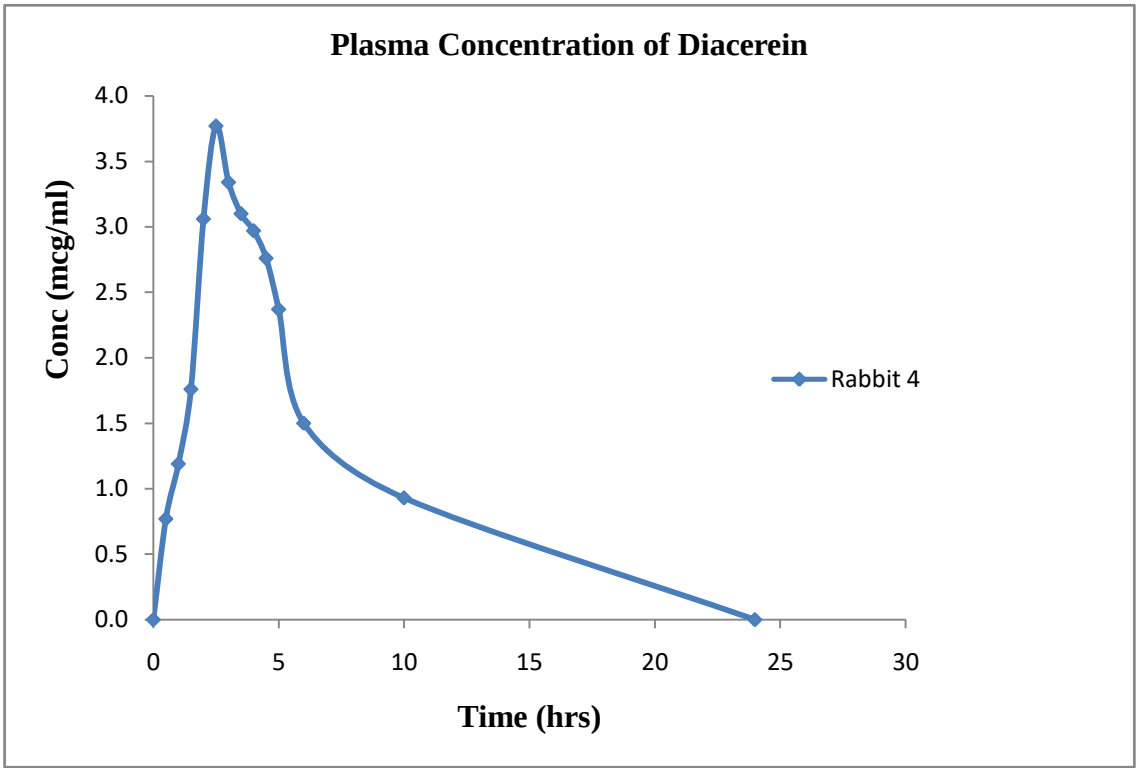


Figure 11.6

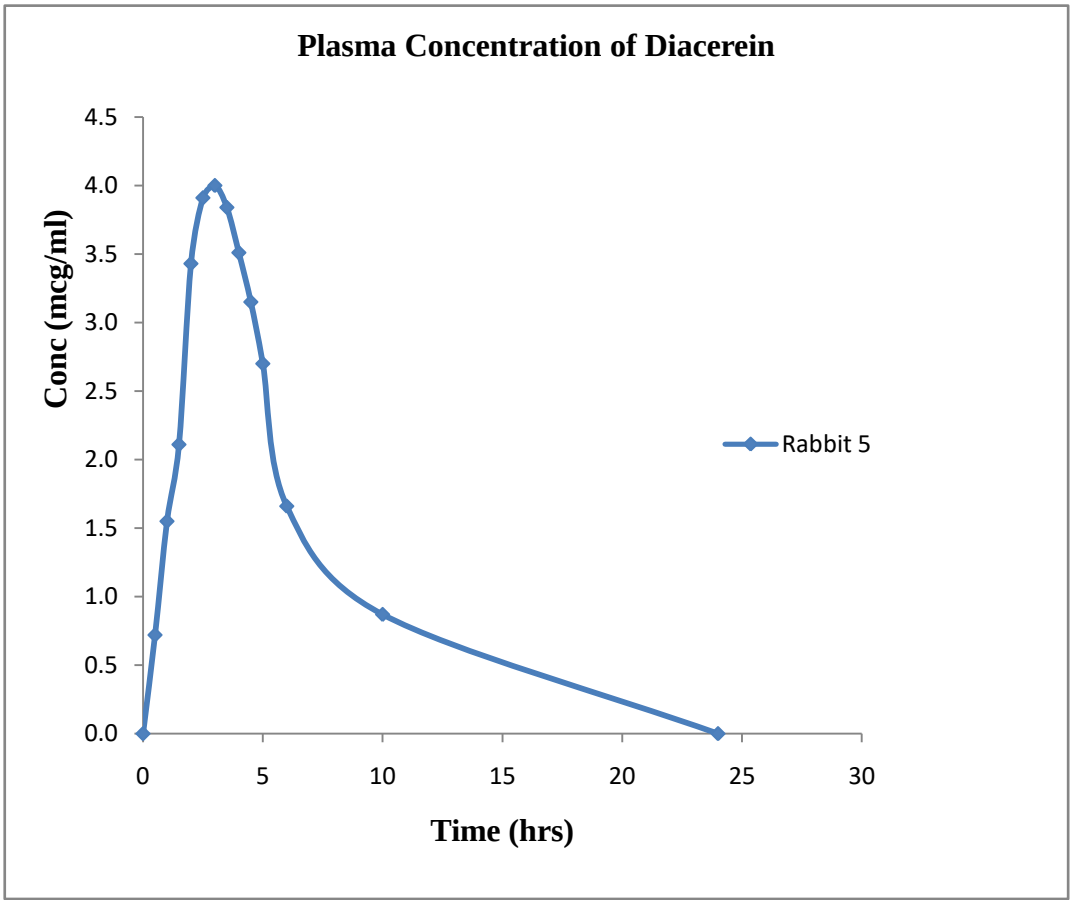


Figure 11.7

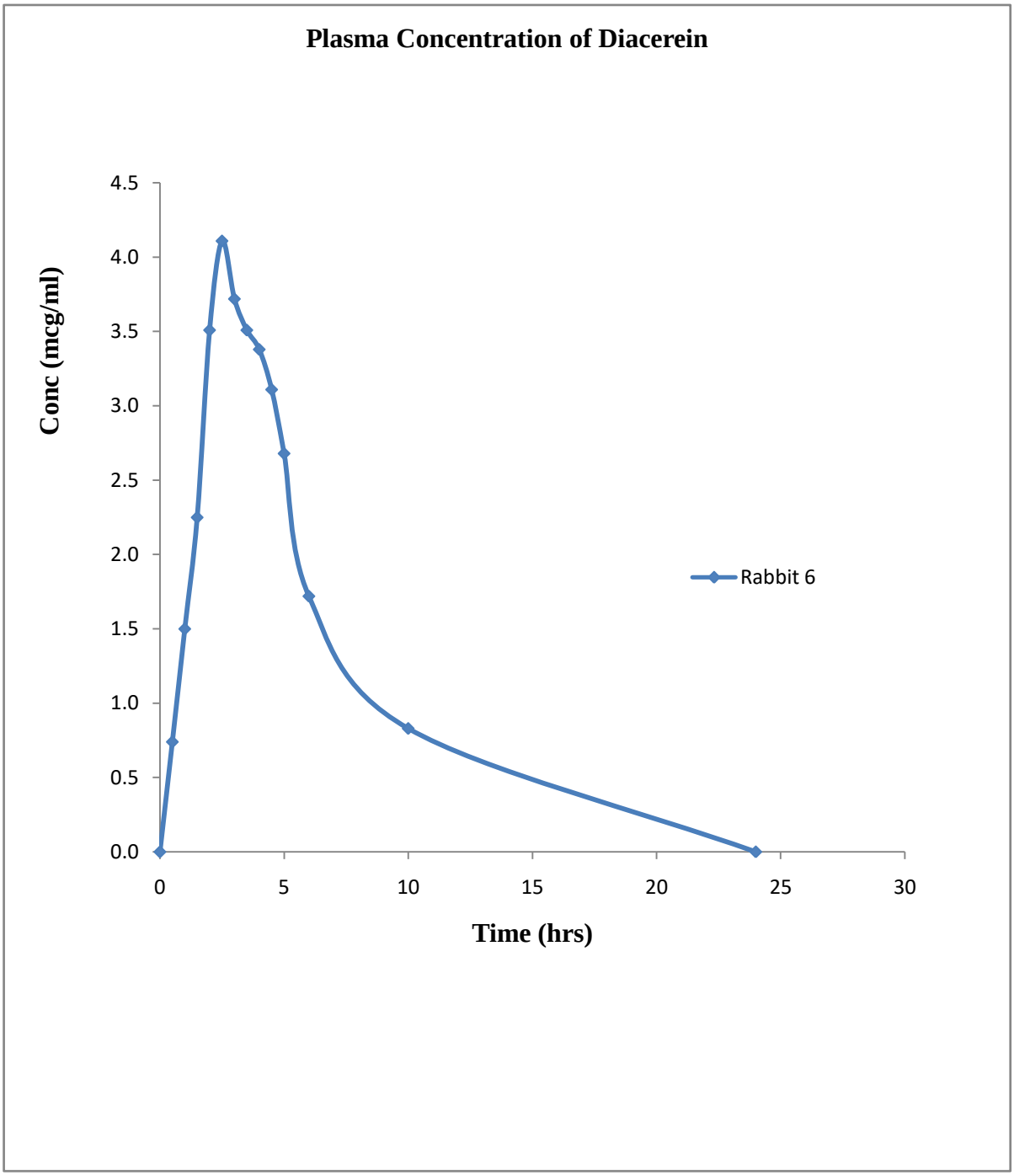

Plasma Concentration of Diacerein
Conc (mcg/ml)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25
30
Time (hrs)
Rabbit 6

Figure 11.8

Table 11.8: Plasma Concentration of Diacerein Microsphere in Rabbit(1-6) Plasma:

	Rabbit-1	Rabbit - 2	Rabbit-3	Rabbit - 4	Rabbit-5	Rabbit - 6	Mean
0.0	0	0	0	0	0	0	0
0.5	0.008	0.009	0.005	0.008	0.004	0.010	0.007
1.0	0.362	0.373	0.364	0.380	0.379	0.369	0.371
1.5	0.504	0.462	0.476	0.471	0.490	0.437	0.473
2.0	1.006	1.020	1.015	1.012	1.003	1.010	1.011
2.5	1.256	1.257	1.253	1.252	1.294	1.242	1.259
3.0	2.047	2.029	2.045	1.939	1.991	2.044	2.016
4.0	**4.054**	**4.038**	**4.020**	**3.990**	**3.962**	**4.031**	4.016
6.0	2.070	2.068	2.061	2.031	2.057	2.023	2.052
8.0	1.910	1.895	1.880	1.854	1.875	2.035	1.908
12.0	0.500	0.451	0.448	0.437	0.421	0.435	0.449
18.0	0.030	0.009	0.046	0.035	0.034	0.030	0.031
24.0	0	0	0	0	0	0	0
C max (mcg/ml)	**4.05**	**4.04**	**4.02**	**3.99**	**3.96**	**4.03**	**4.02**
T max (hrs.)	**4.0**	**4.0**	**4.0**	**4.0**	**4.0**	**4.0**	**4.00**
Auc $_{0-t}$ (mcg-hr/ml)	**21.64**	**21.24**	**21.26**	**20.91**	**20.97**	**21.53**	**21.26**
Auc $_{0-\infty}$ (mcg-hr/ml)	**21.73**	**21.26**	**21.40**	**21.02**	**21.07**	**21.62**	**21.35**
K_{el}(hr-1)	**0.346**	**0.430**	**0.318**	**0.336**	**0.339**	**0.348**	**0.35**
T $_{1/2}$ (hrs.)	**2.00**	**1.61**	**2.18**	**2.06**	**2.04**	**1.99**	**1.98**

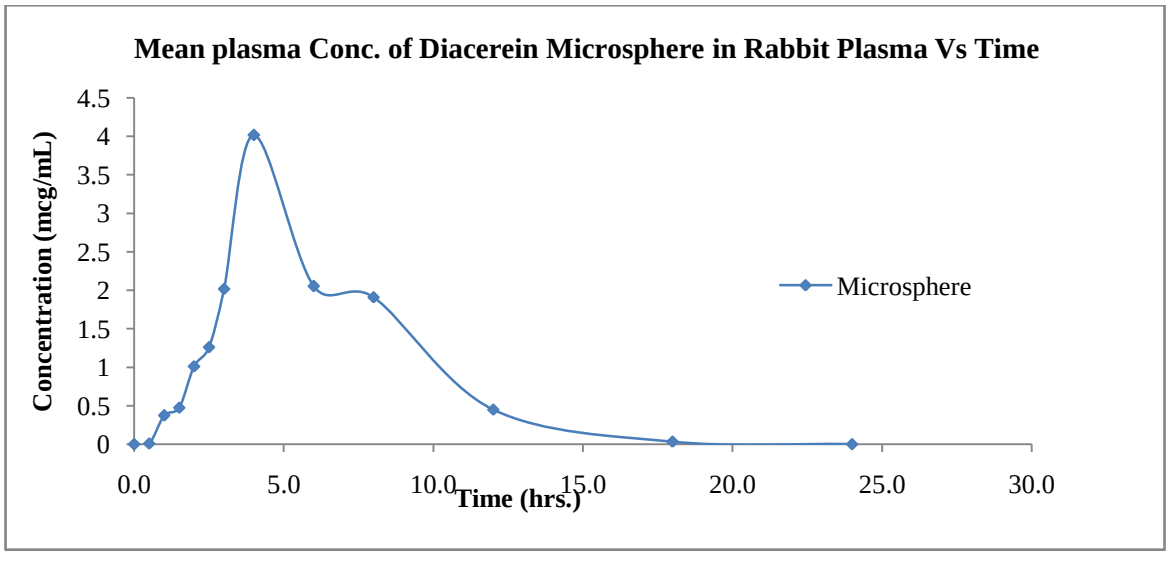


Figure 11.9

Graphical Representation of Blood Plasma Concentration of Diacerein microsphere in rabbit (1-6)

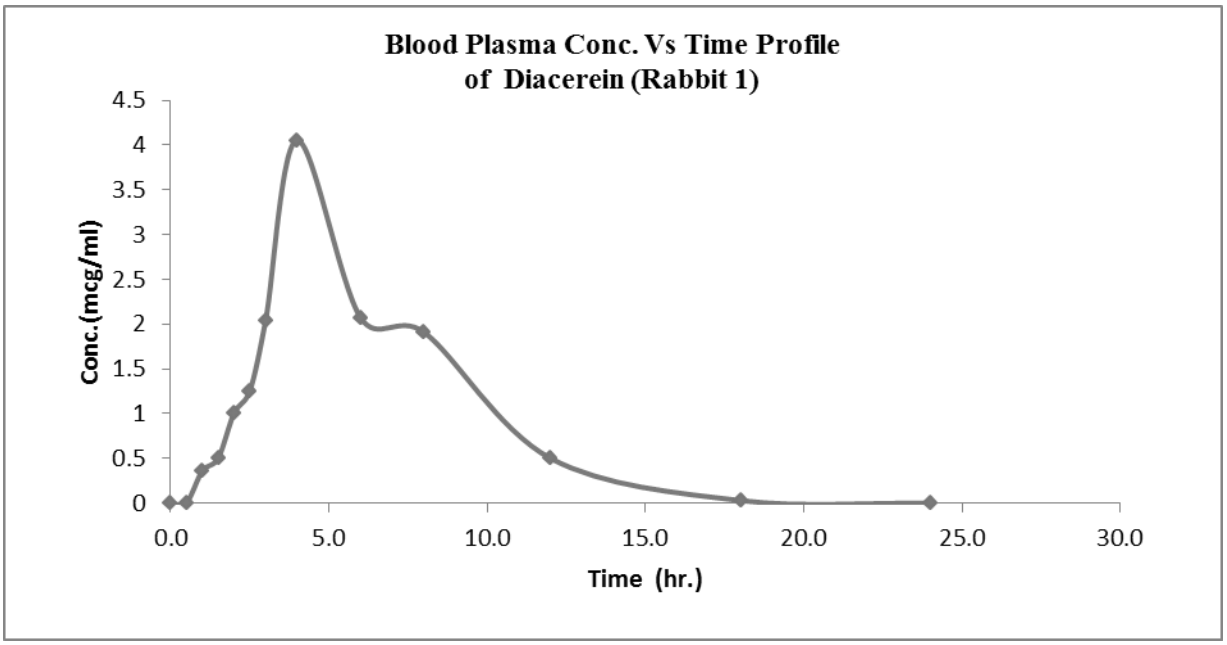


Figure 11.10

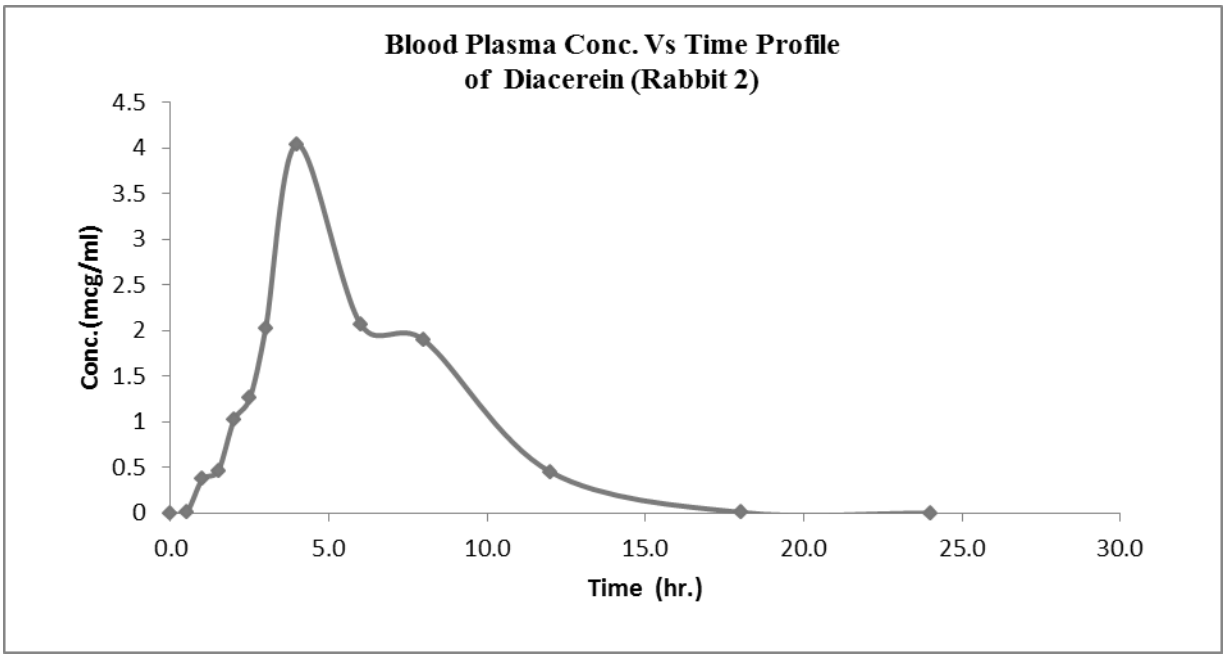


Figure 11.11

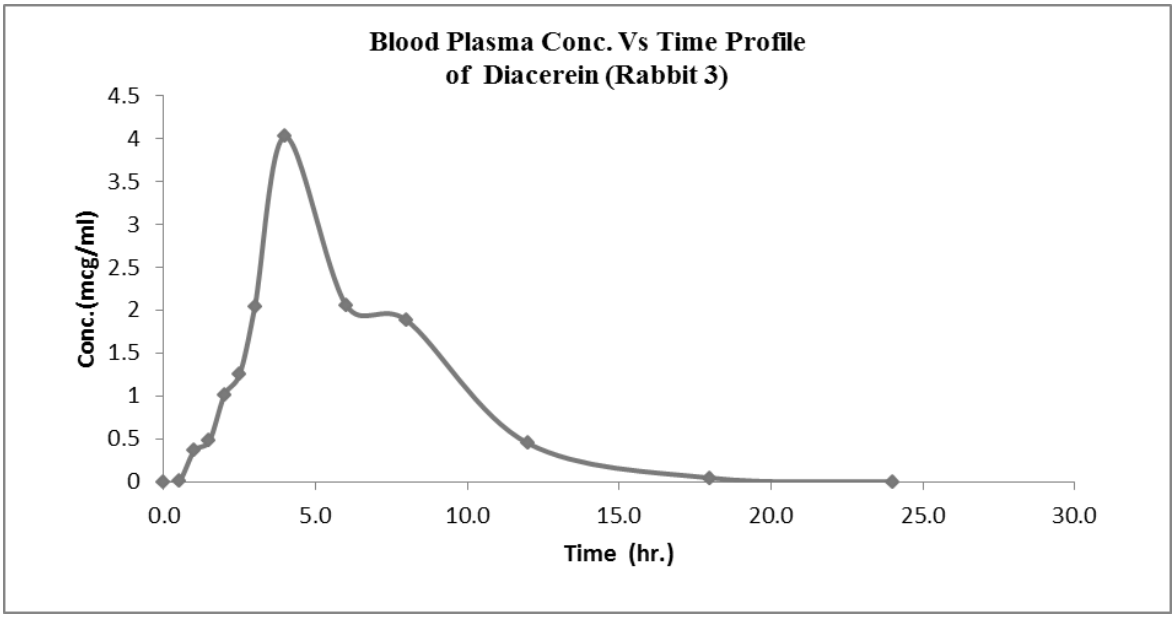


Figure 11.12

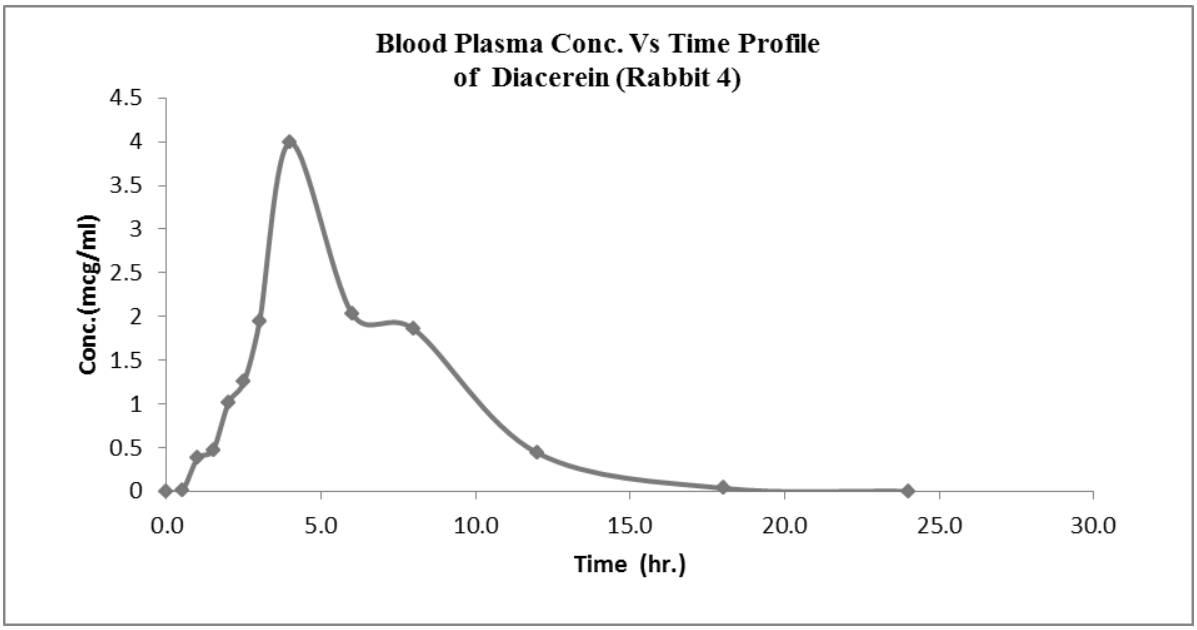


Figure 11.13

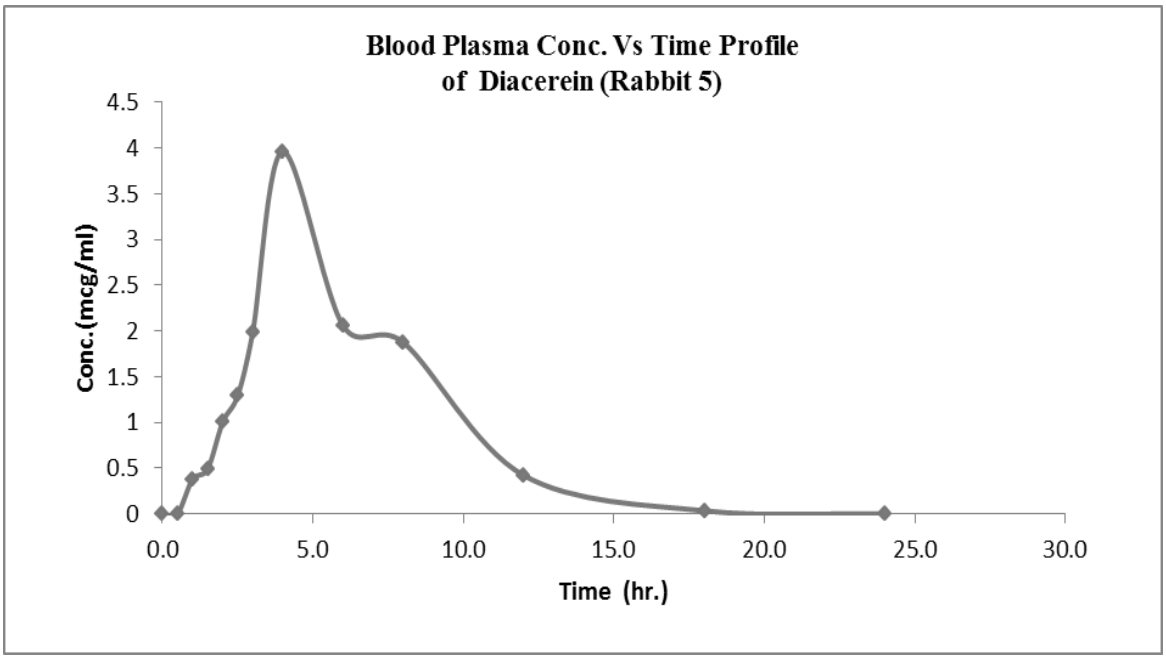


Figure 11.14

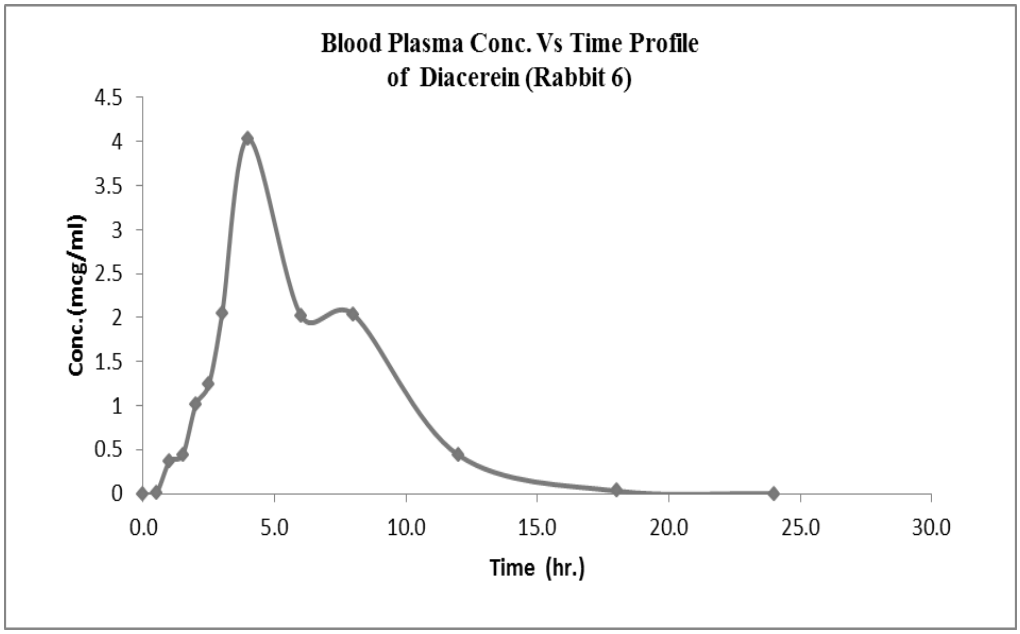


Figure 11.15

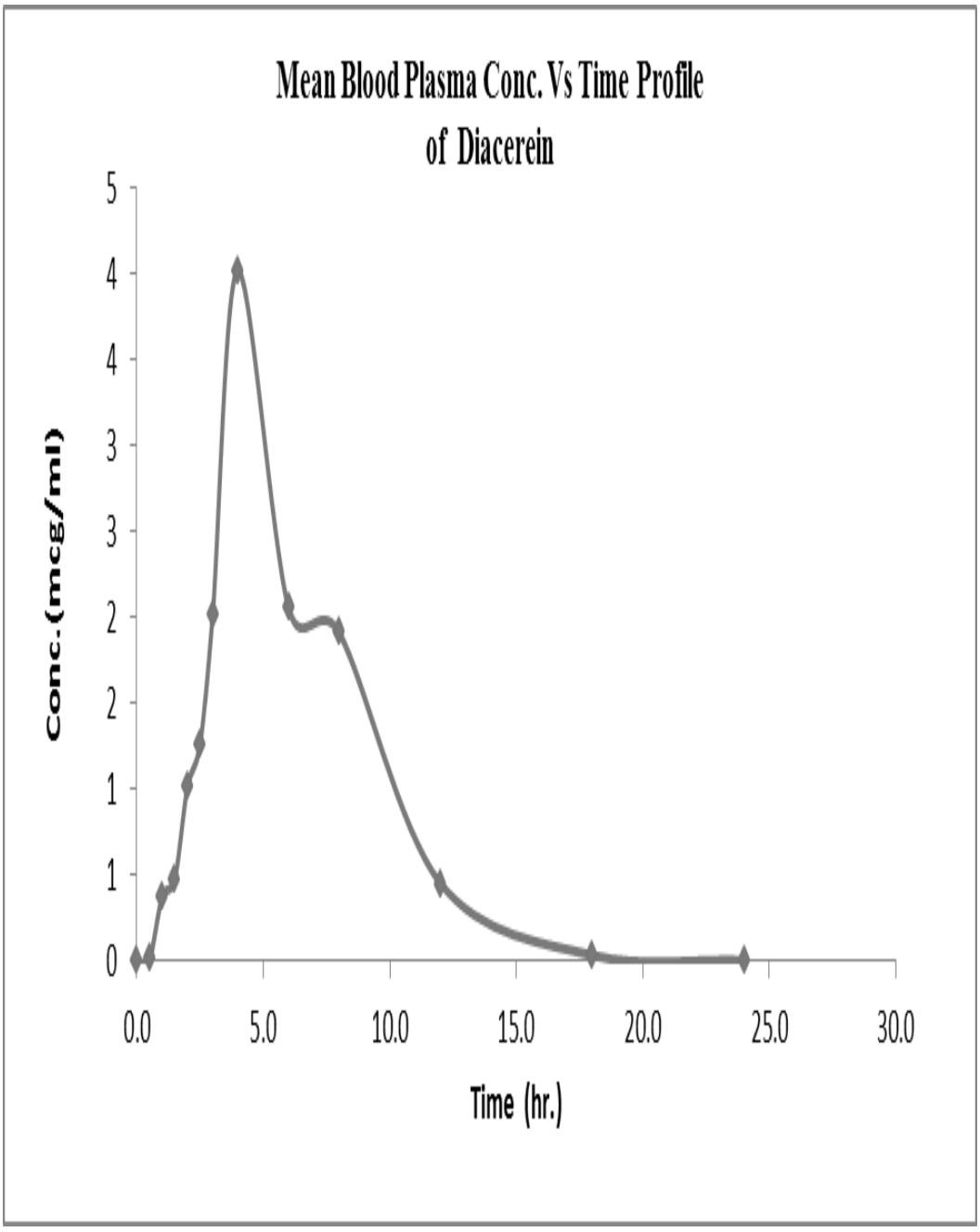


Figure 11.16

DCN Microsphere

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by:	Admin
Sample name:	Calibration 0.37mcg/ml
Sample ID:	Calibration
Injection volume:	20µl
Method File:	Calibration

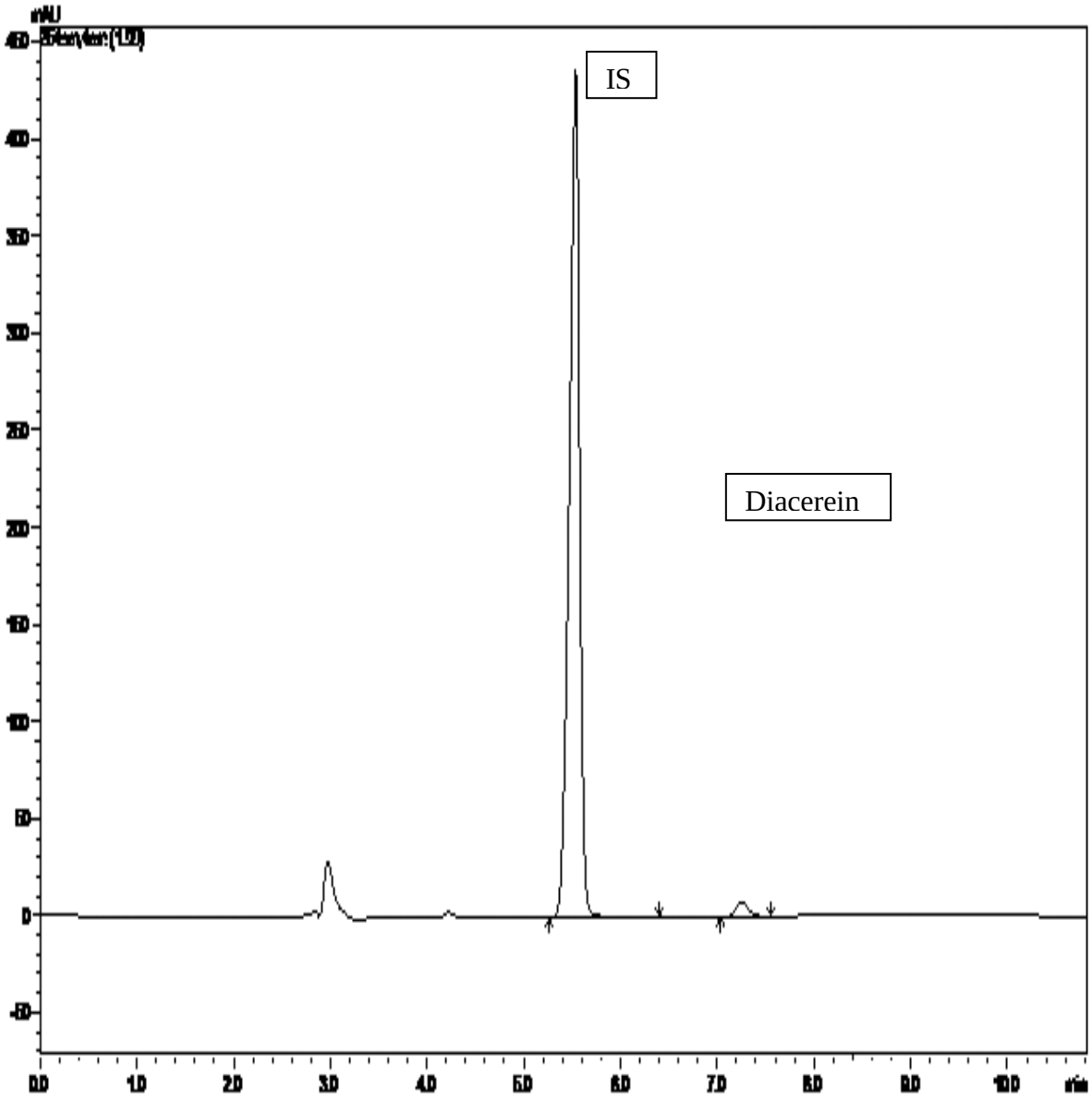


Figure 11.17

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by:	Admin
Sample name:	Calibration 0.5mcg/ml
Sample ID:	Calibration
Injection volume:	20µl
Method File:	Calibration

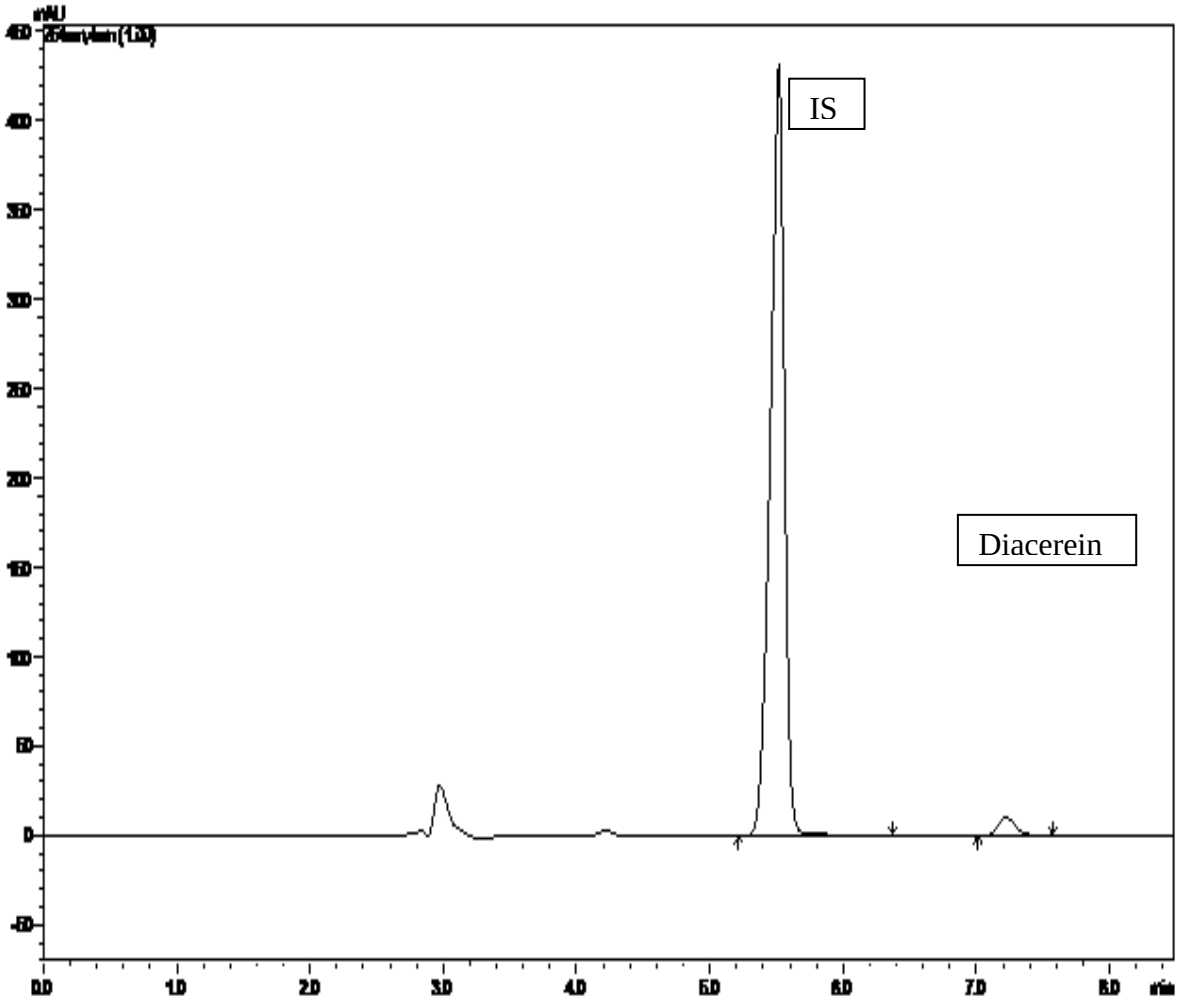


Figure 11.18

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: Calibration 1mcg/ml
Sample ID: Calibration
Injection volume: 20µl
Method File: Calibration

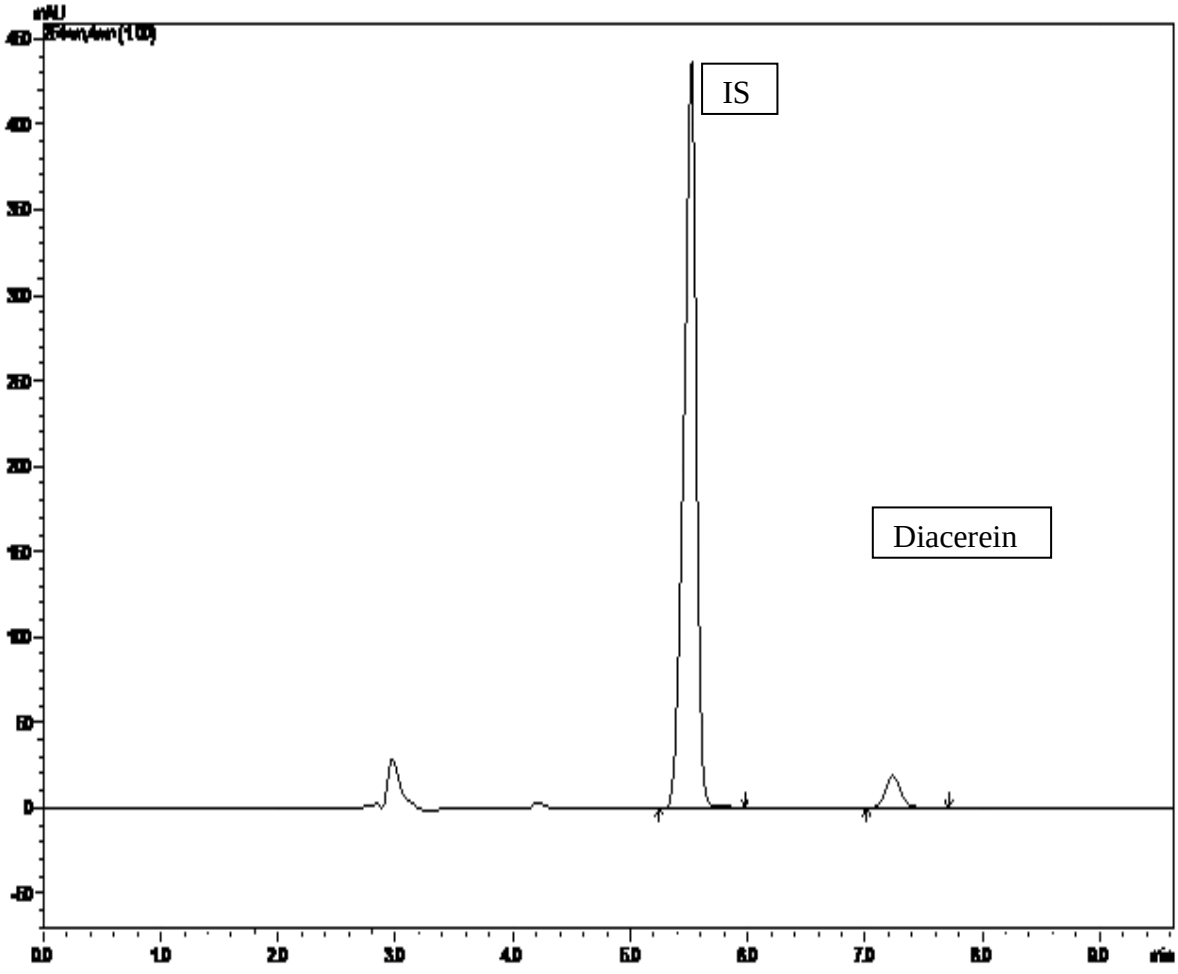


Figure 11.19

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by:	Admin
Sample name:	Calibration 2mcg/ml
Sample ID:	Calibration
Injection volume:	20µl
Method File:	Calibration

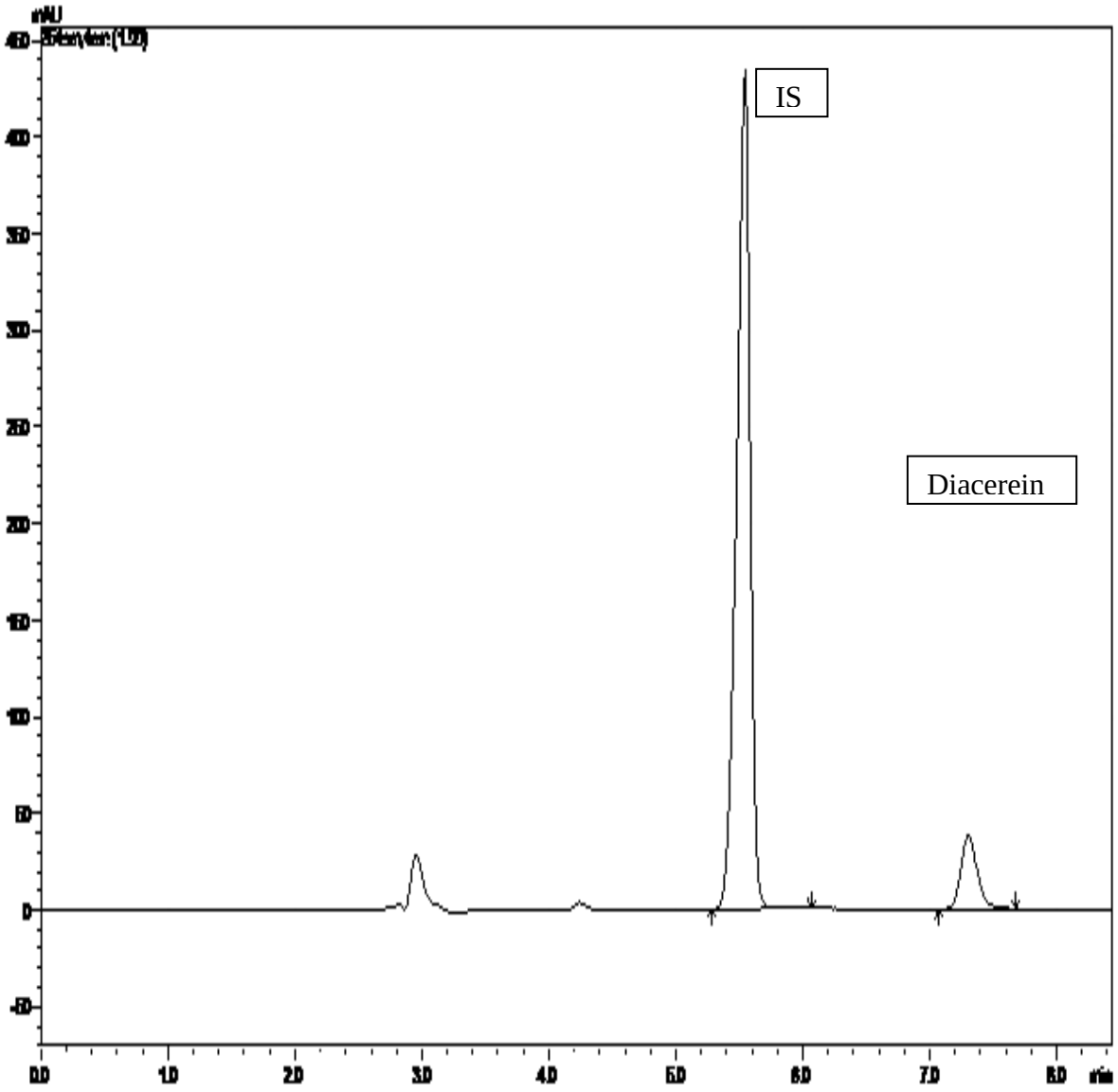


Figure 11.20

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: Calibration 4mcg/ml
Sample ID: Calibration
Injection volume: 20µl
Method File: Calibration

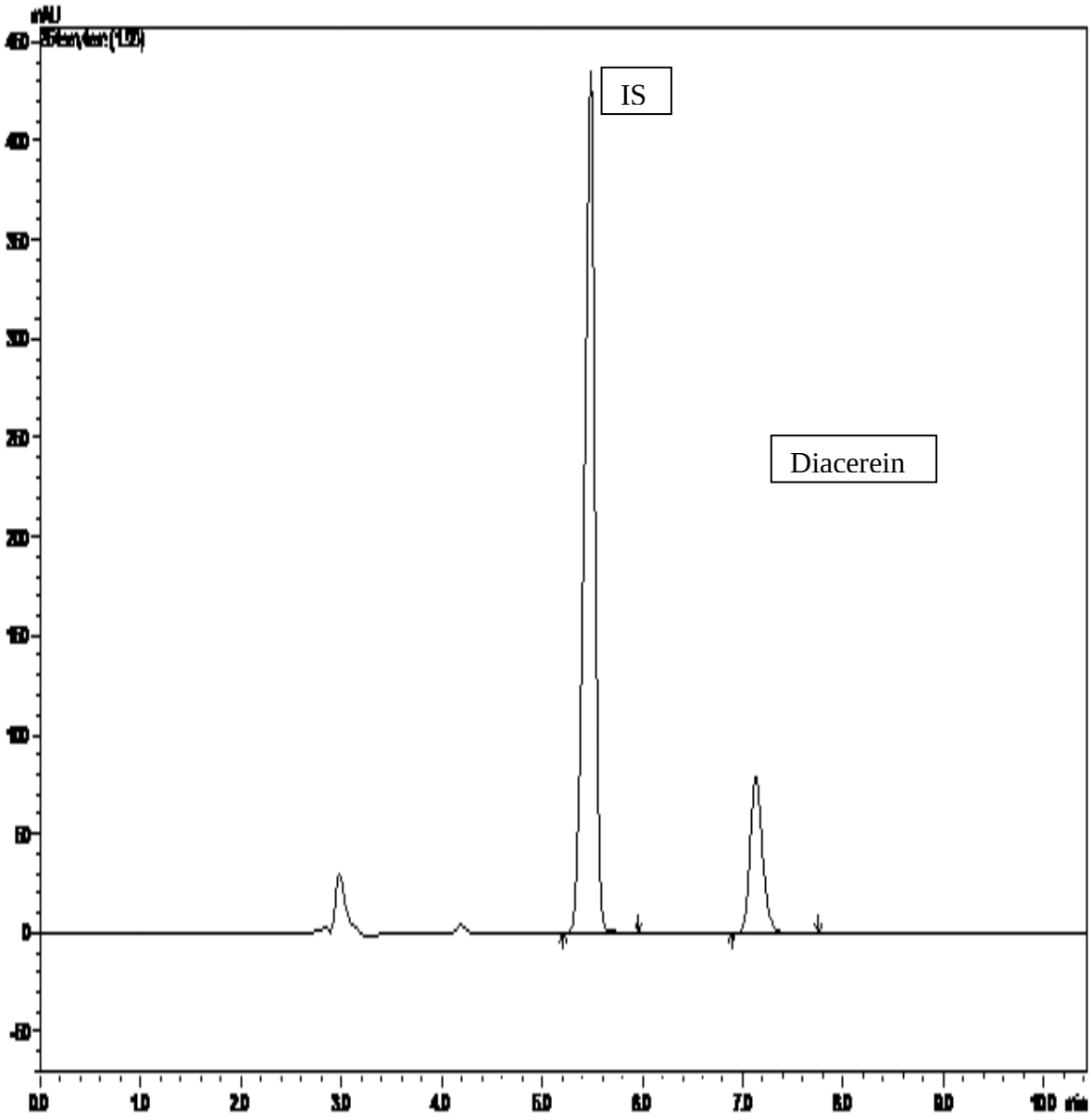


Figure 11.21

D:/Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: Calibration 8mcg/ml
Sample ID: Calibration
Injection volume: 20µl
Method File: Calibration

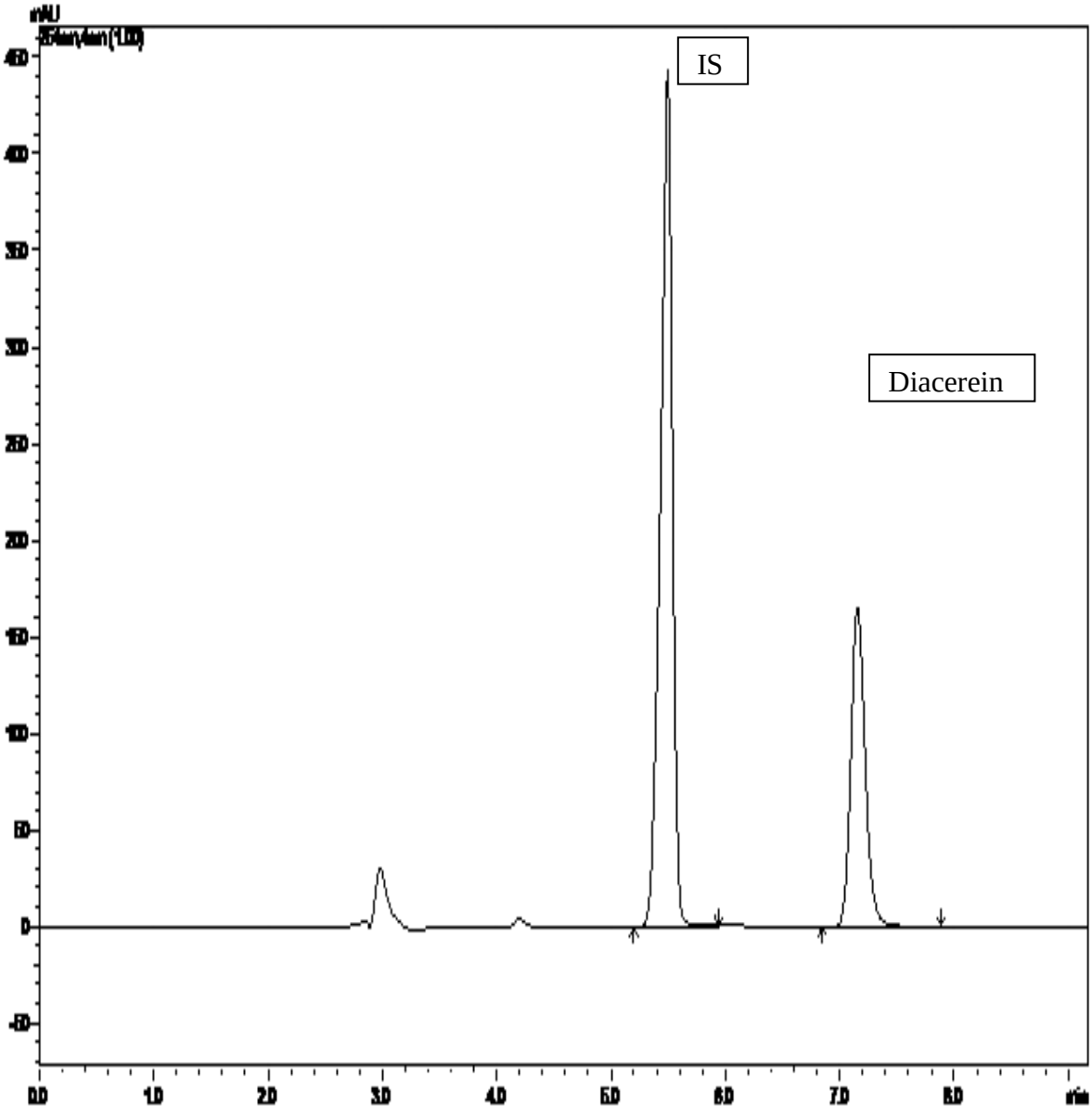


Figure 11.22

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: Calibration 10mcg/ml
Sample ID: Calibration
Injection volume: 20µl
Method File: Calibration

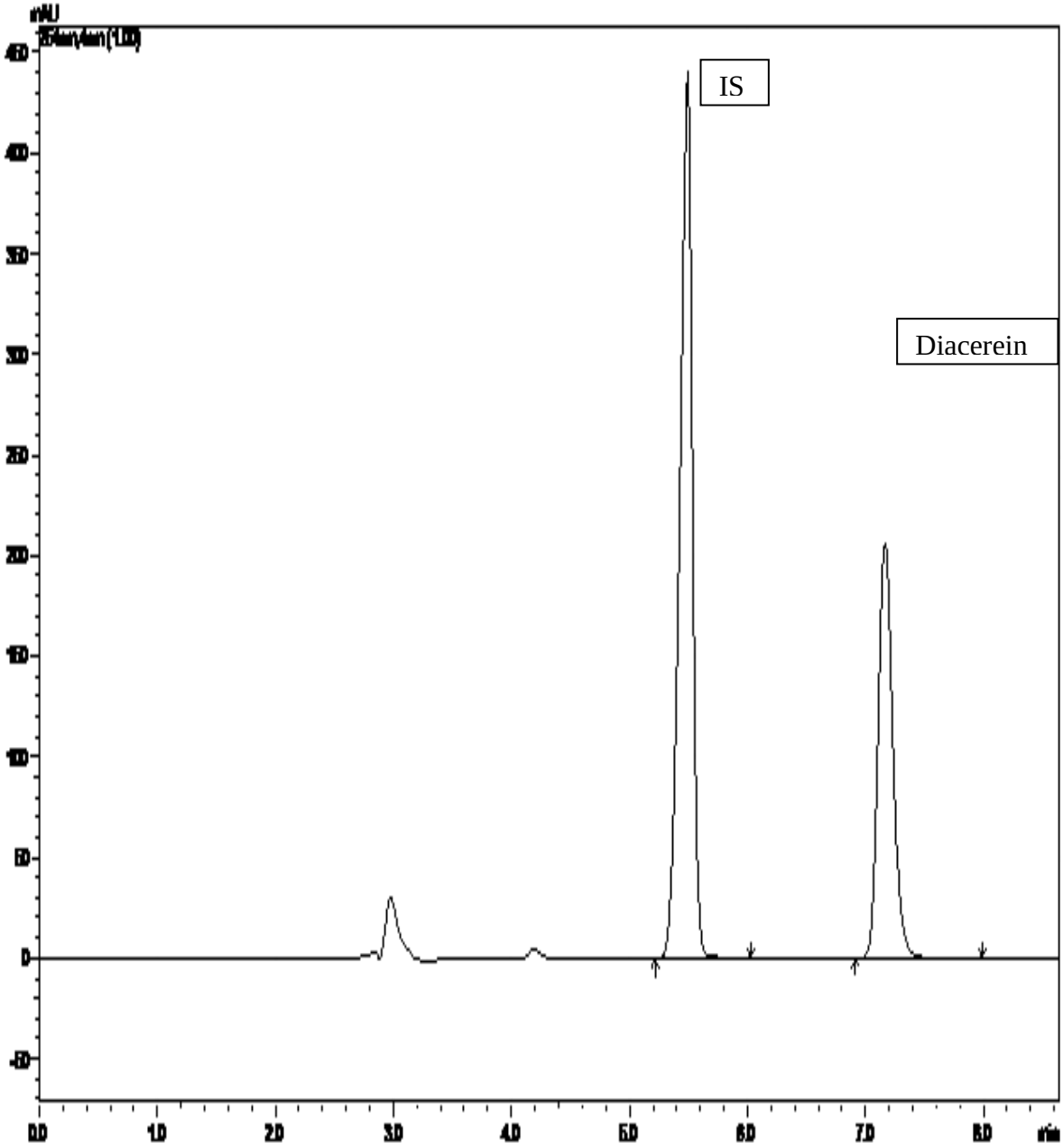


Figure 11.23

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: Calibration 12mcg/ml
Sample ID: Calibration
Injection volume: 20µl
Method File: Calibration

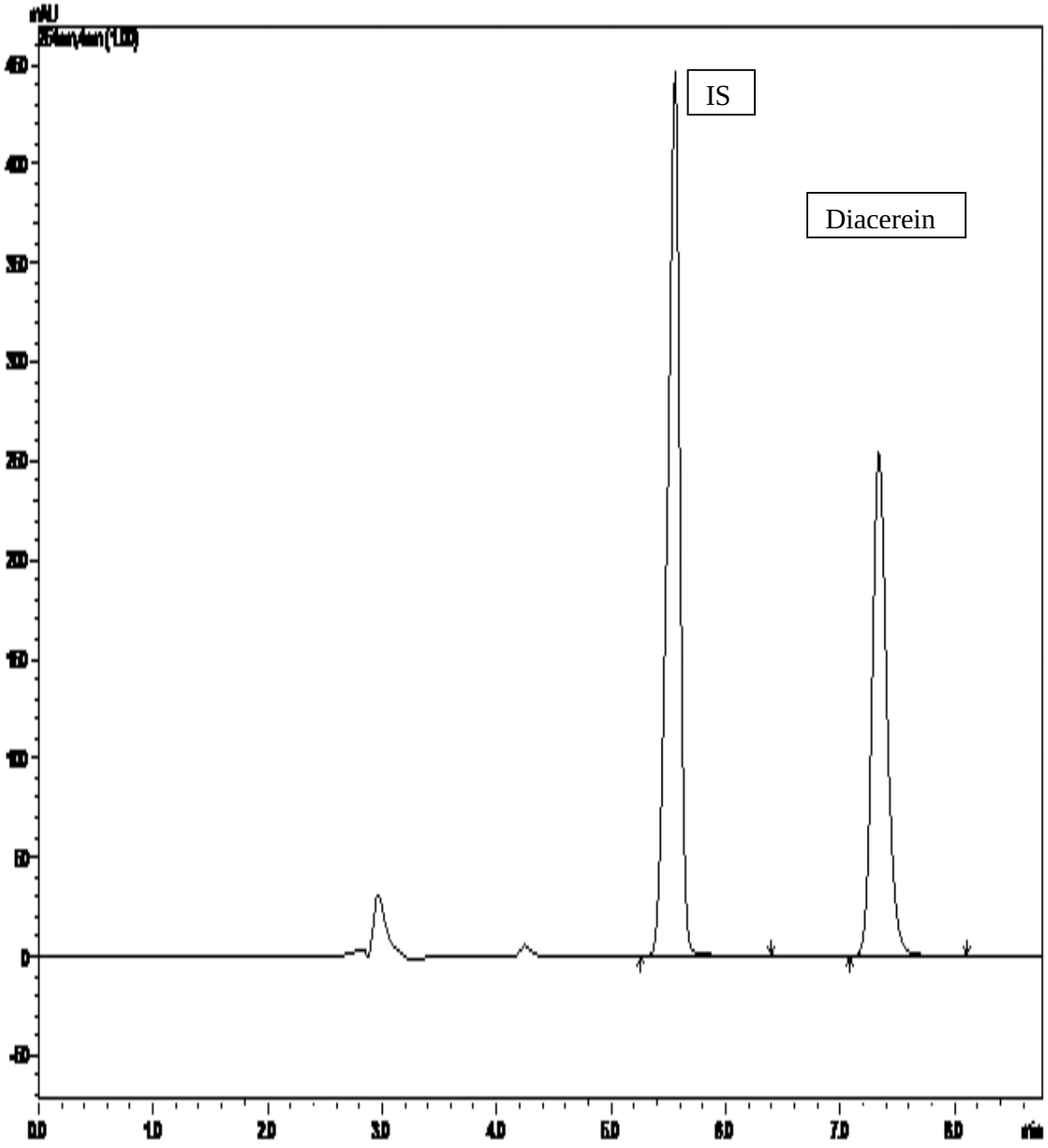


Figure 11.24

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: QC
Sample ID: LLOQ
Injection volume: 20µl
Method File: Calibration

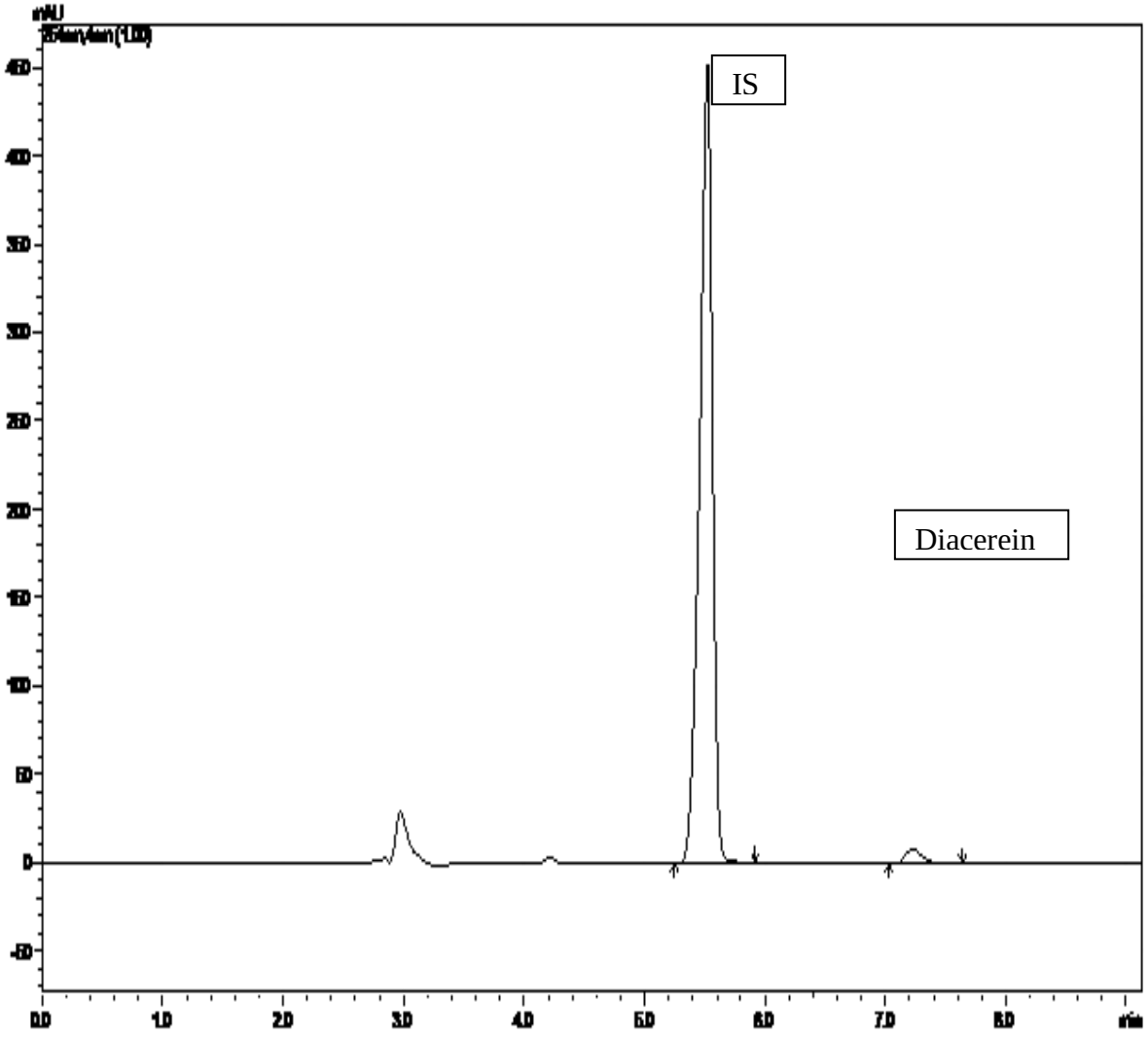


Figure 11.25

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by:	Admin
Sample name:	QC
Sample ID:	LQC
Injection volume:	20µl
Method File:	Calibration

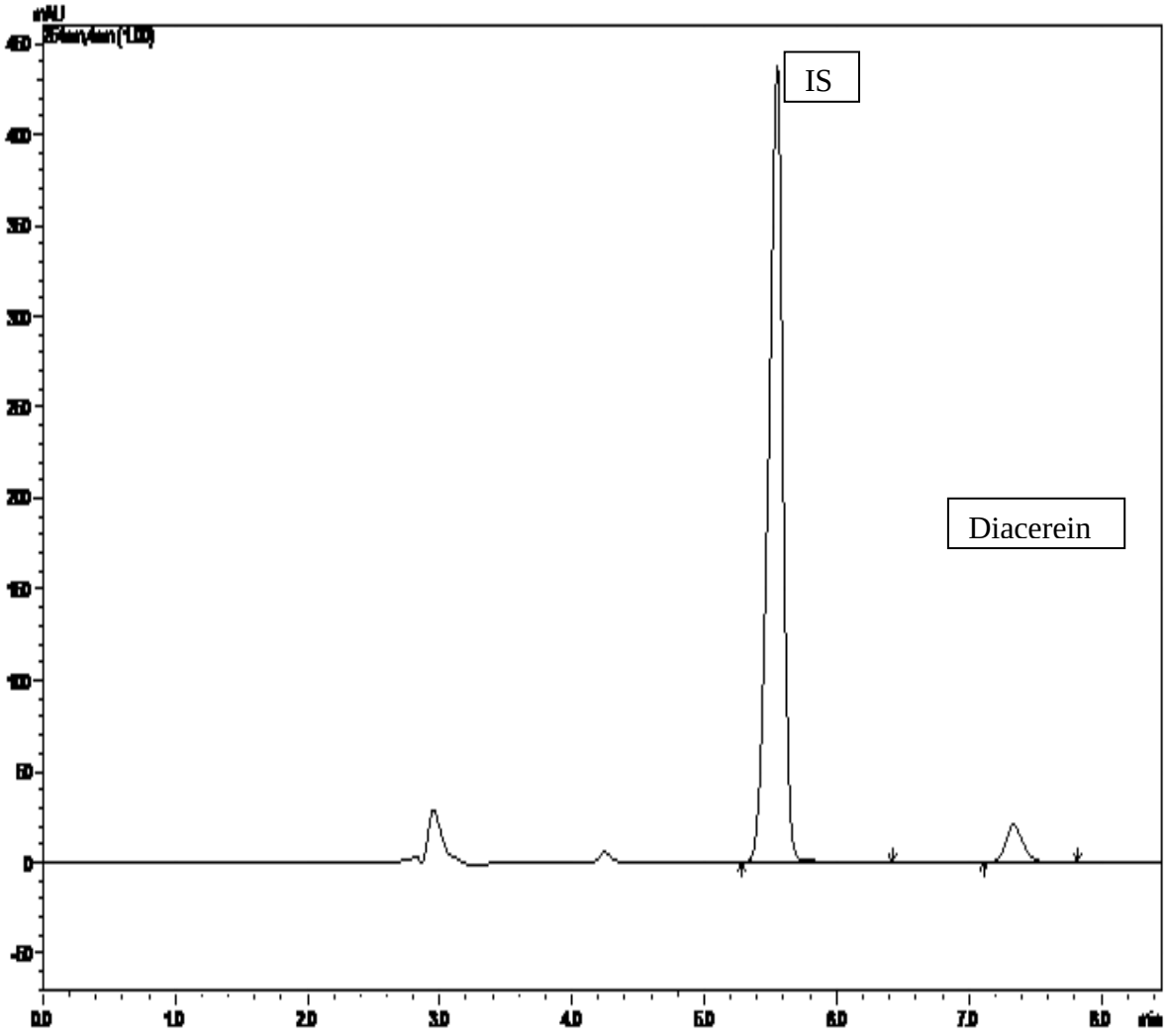


Figure 11.26

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: QC
Sample ID: MQC
Injection volume: 20µl
Method File: Calibration

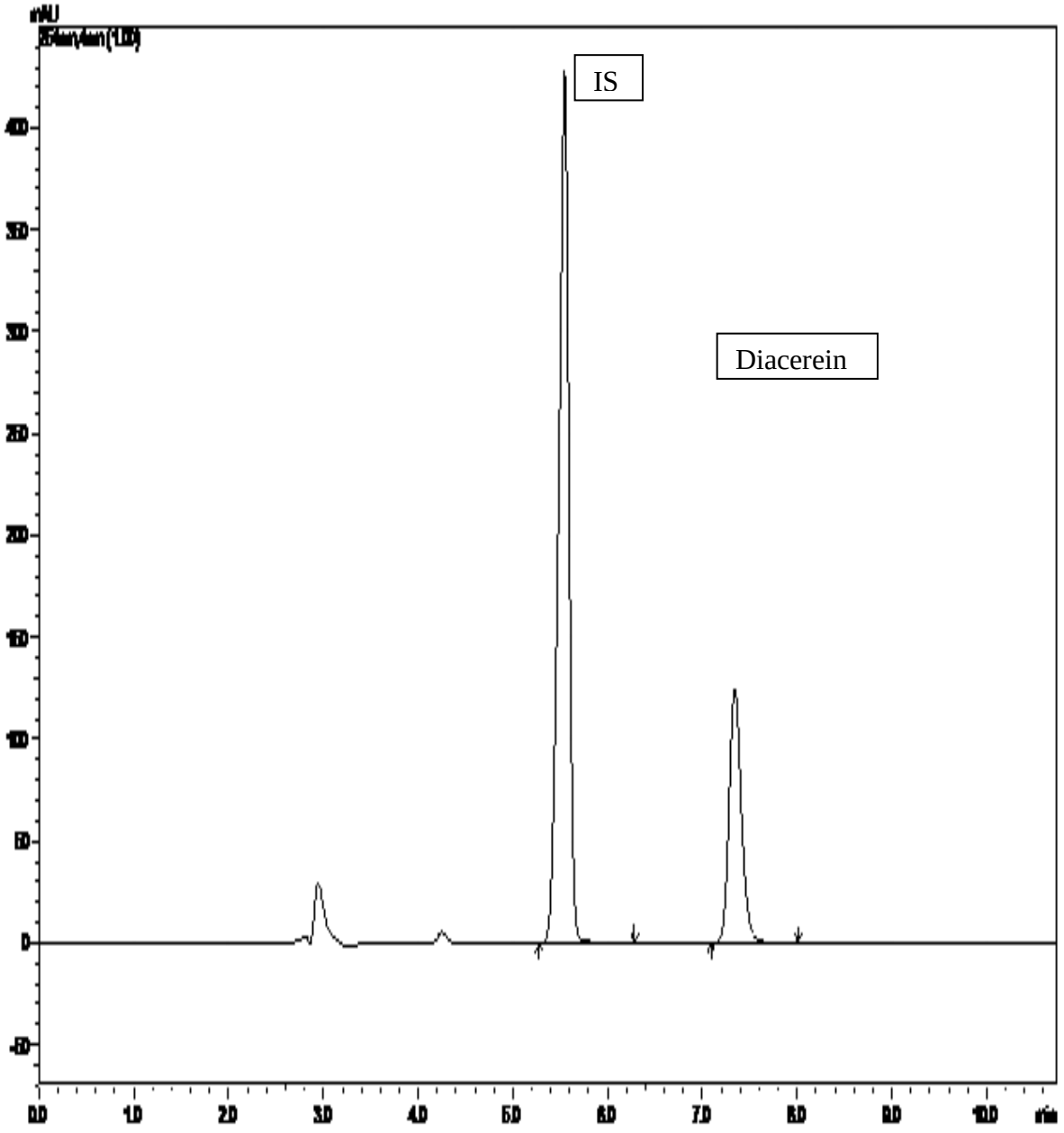


Figure 11.27

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: QC
Sample ID: HQC
Injection volume: 20µl
Method File: Calibration

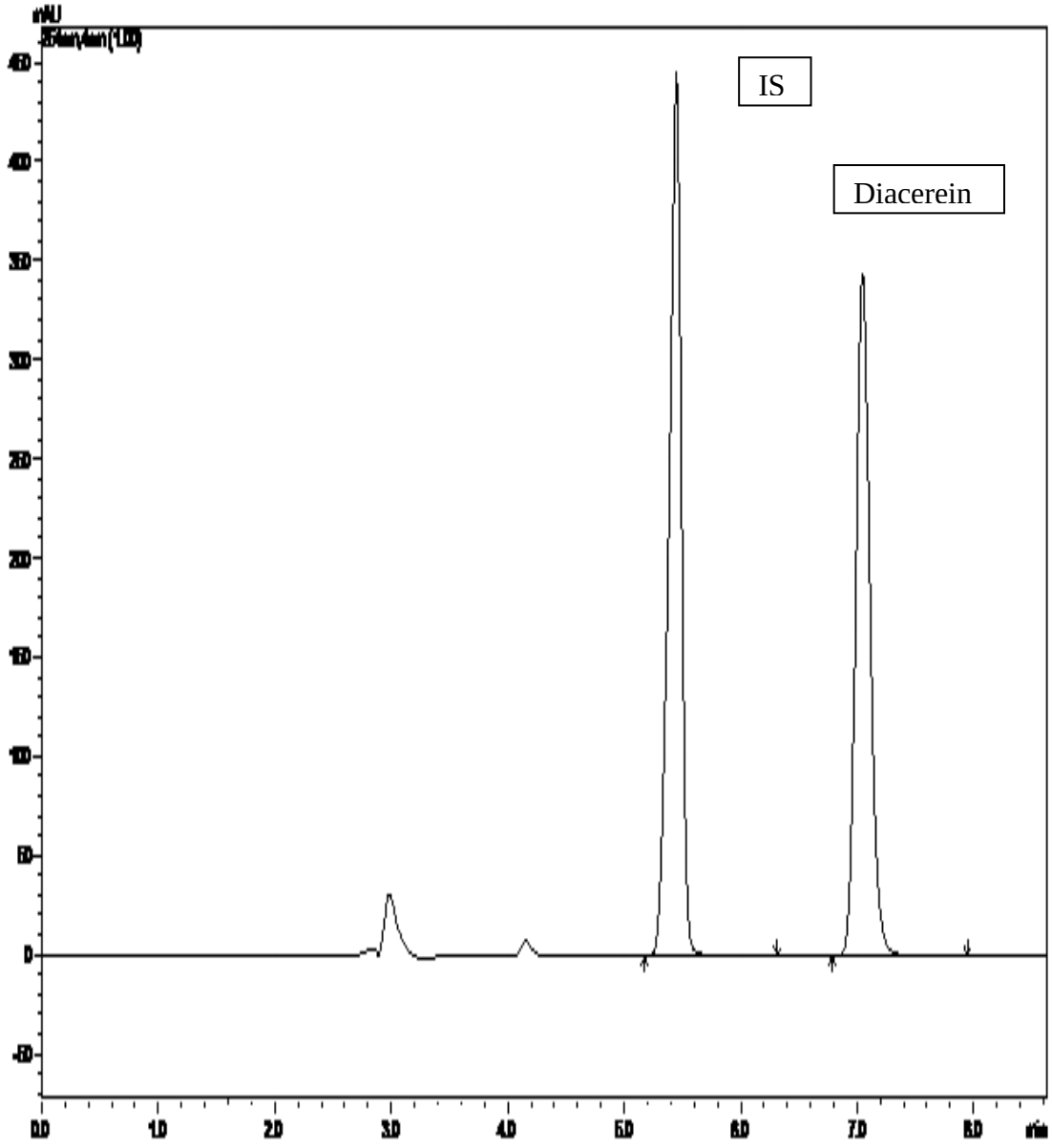


Figure 11.28

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: QC
Sample ID: LQC Freeze Thaw
Injection volume: 20µl
Method File: Calibration

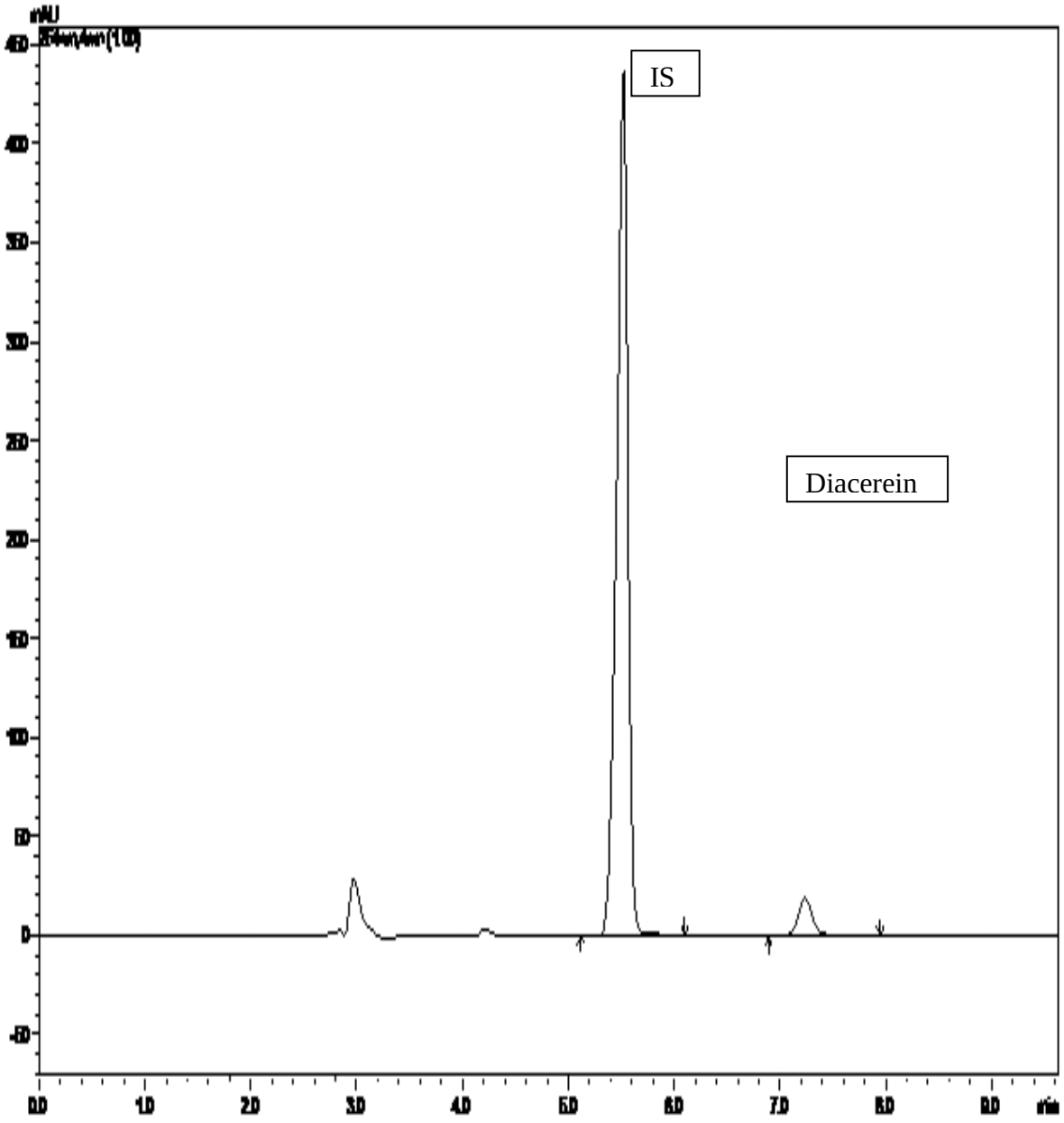


Figure 11.29

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: QC
Sample ID: LQC Freshly Thaw
Injection volume: 20µl
Method File: Calibration

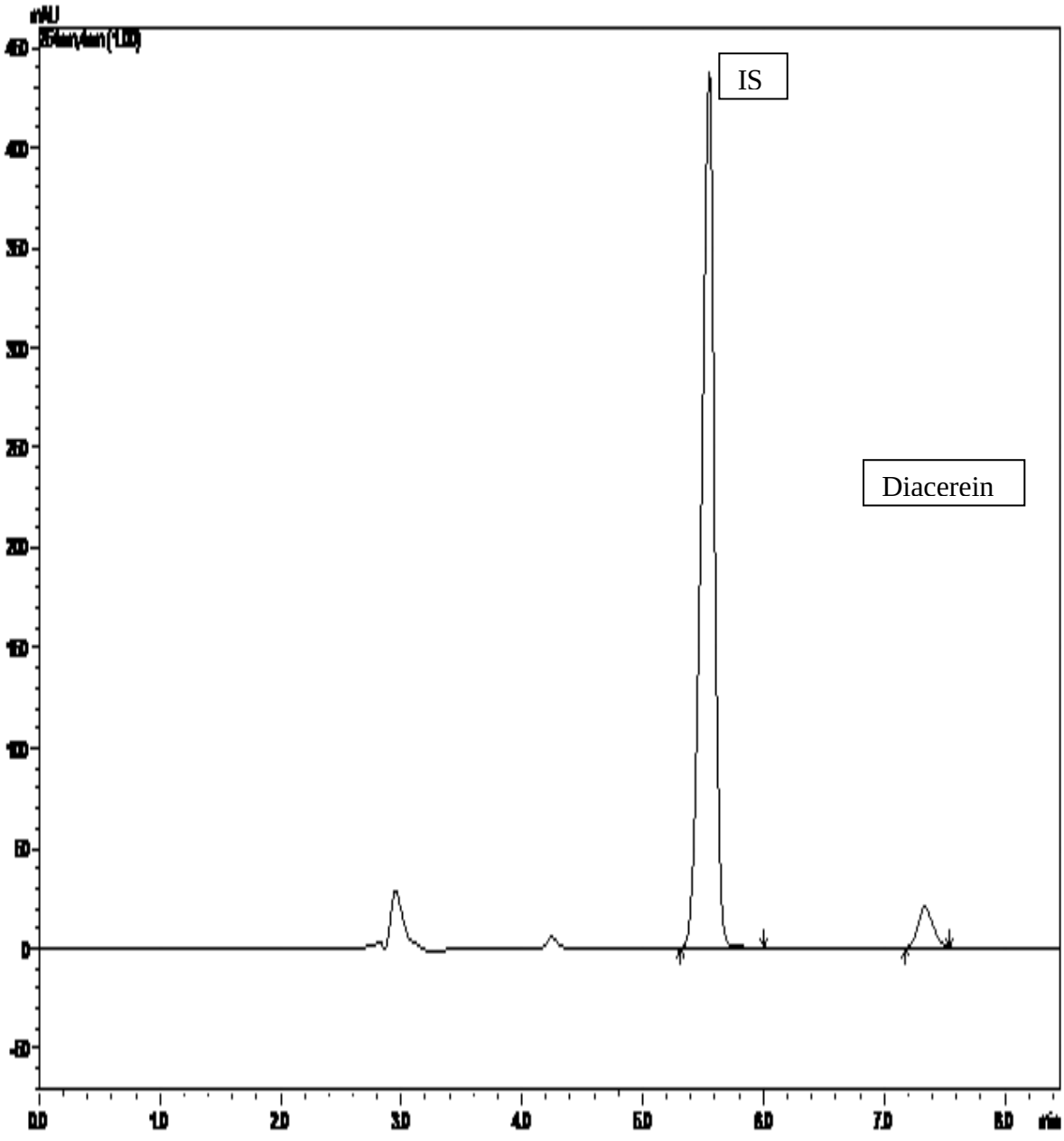


Figure 11.30

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: QC
Sample ID: LQC in Plasma
Injection volume: 20µl
Method File: Calibration

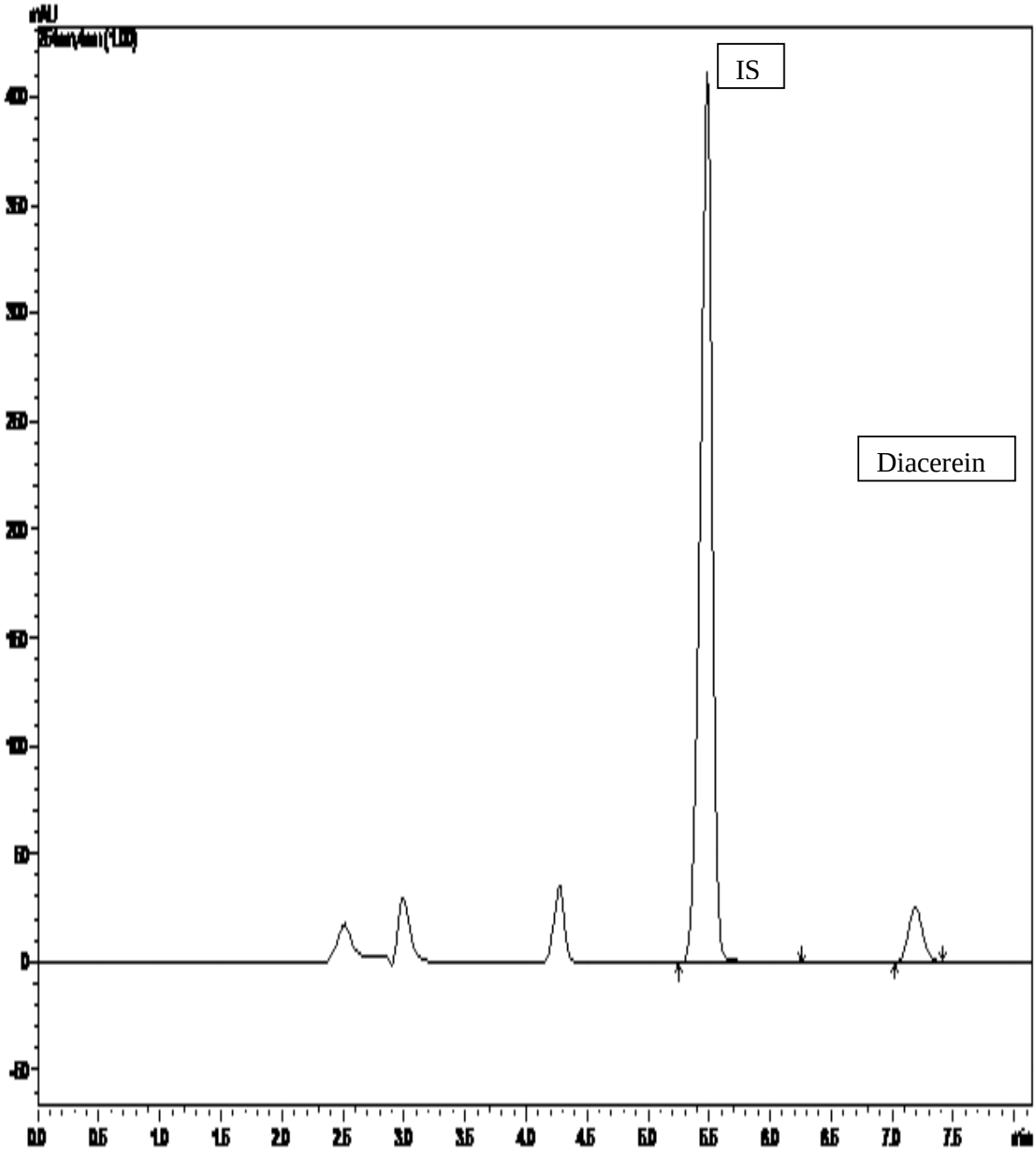


Figure 11.31

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: QC
Sample ID: LQC in Diluents
Injection volume: 20µl
Method File: Calibration

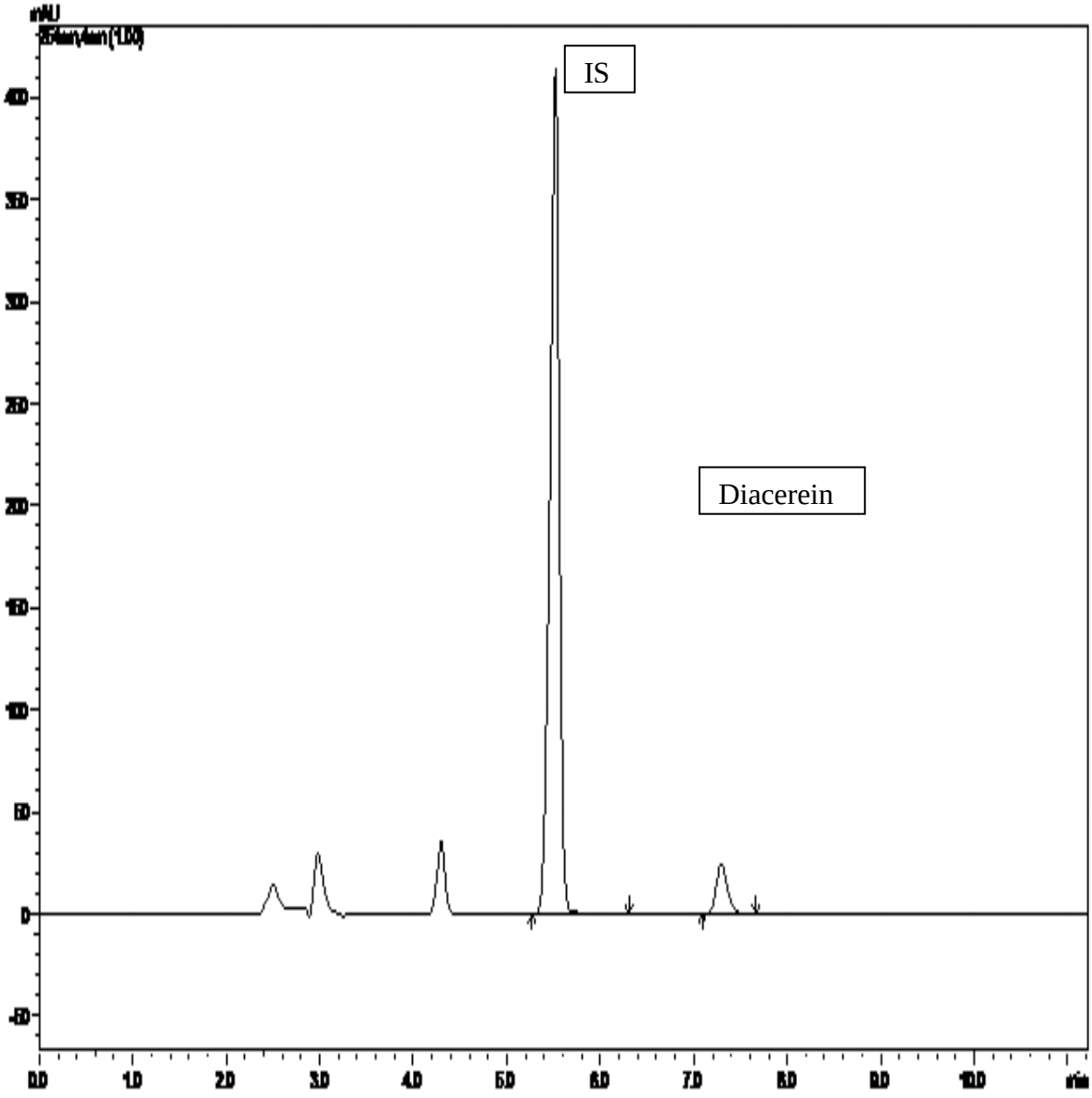


Figure 11.32

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: QC
Sample ID: MQC Freeze Thaw
Injection volume: 20µl
Method File: Calibration

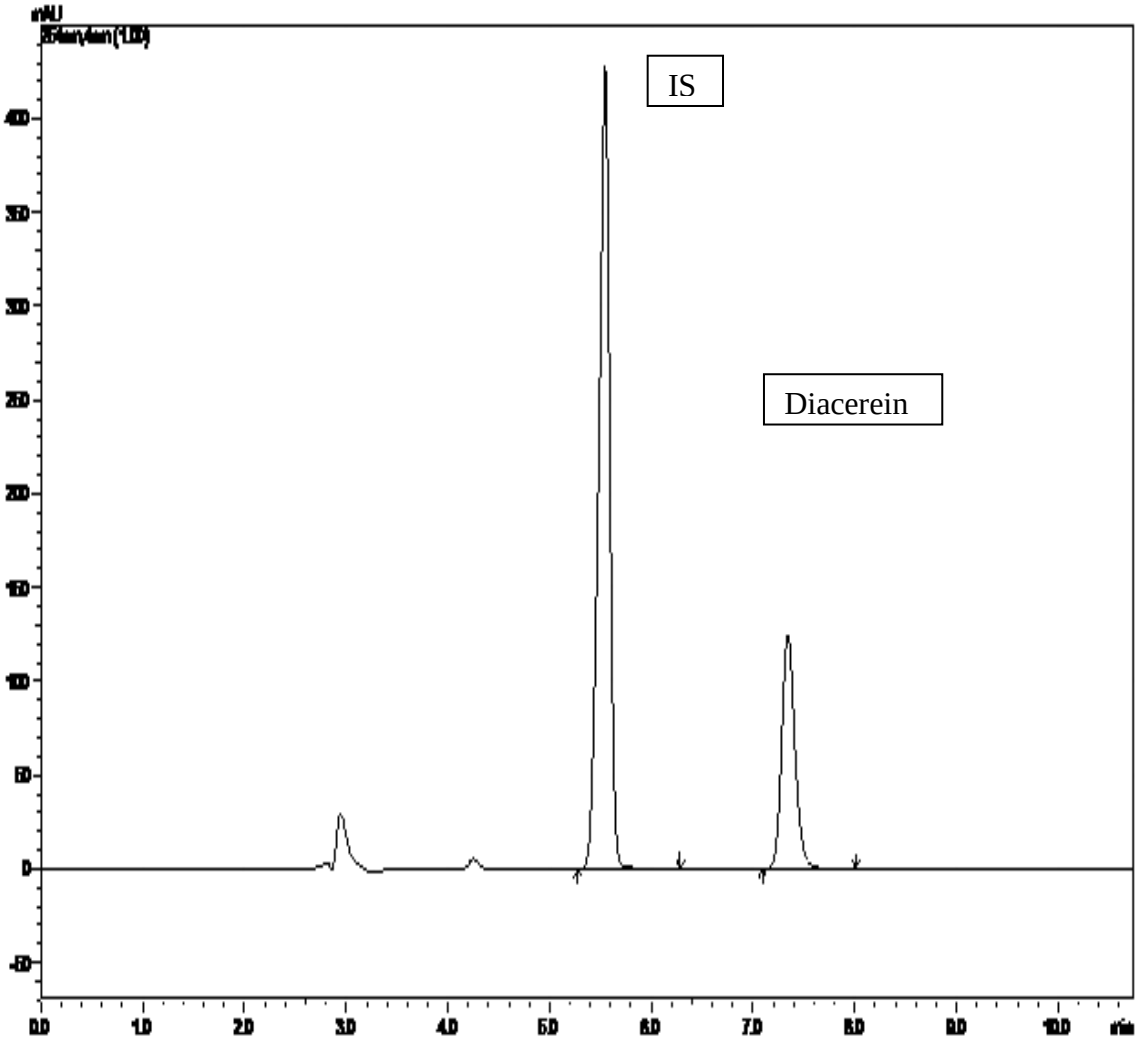


Figure 11.33

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: QC
Sample ID: MQC Freshly Thaw
Injection volume: 20µl
Method File: Calibration

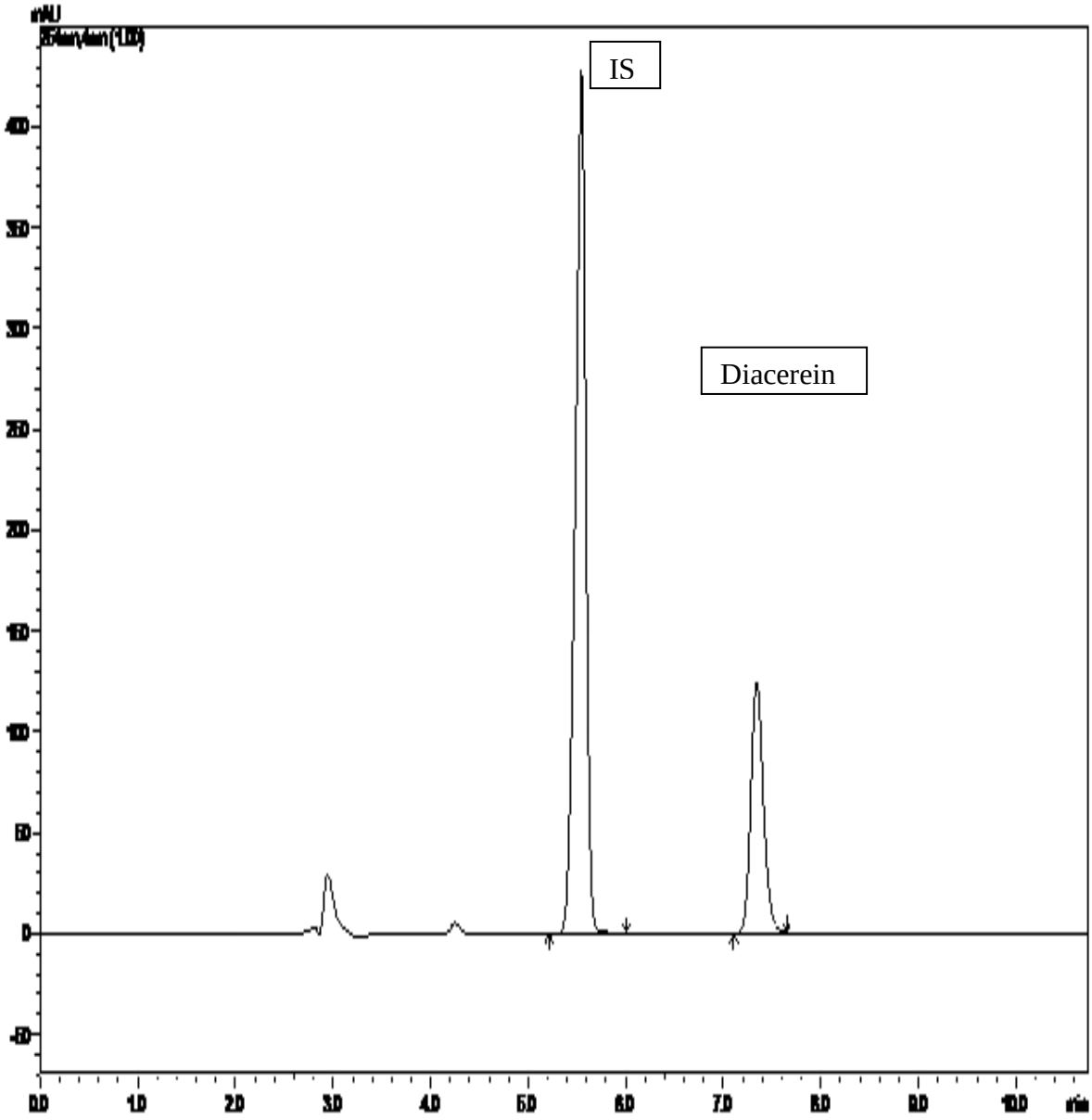


Figure 11.34

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: QC
Sample ID: MQC in Plasma
Injection volume: 20µl
Method File: Calibration

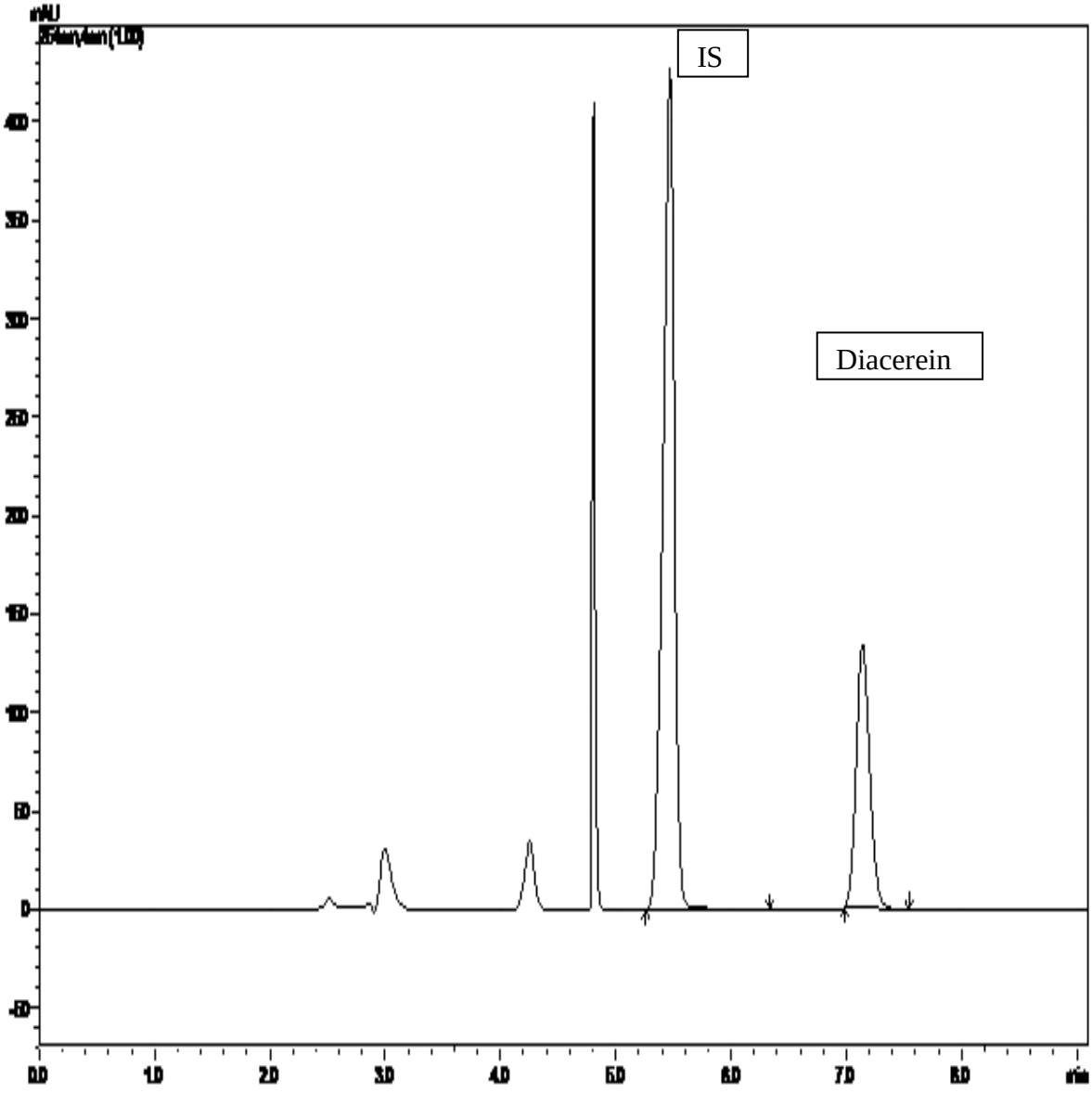


Figure 11.35

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: QC
Sample ID: MQC in Diluent
Injection volume: 20µl
Method File: Calibration

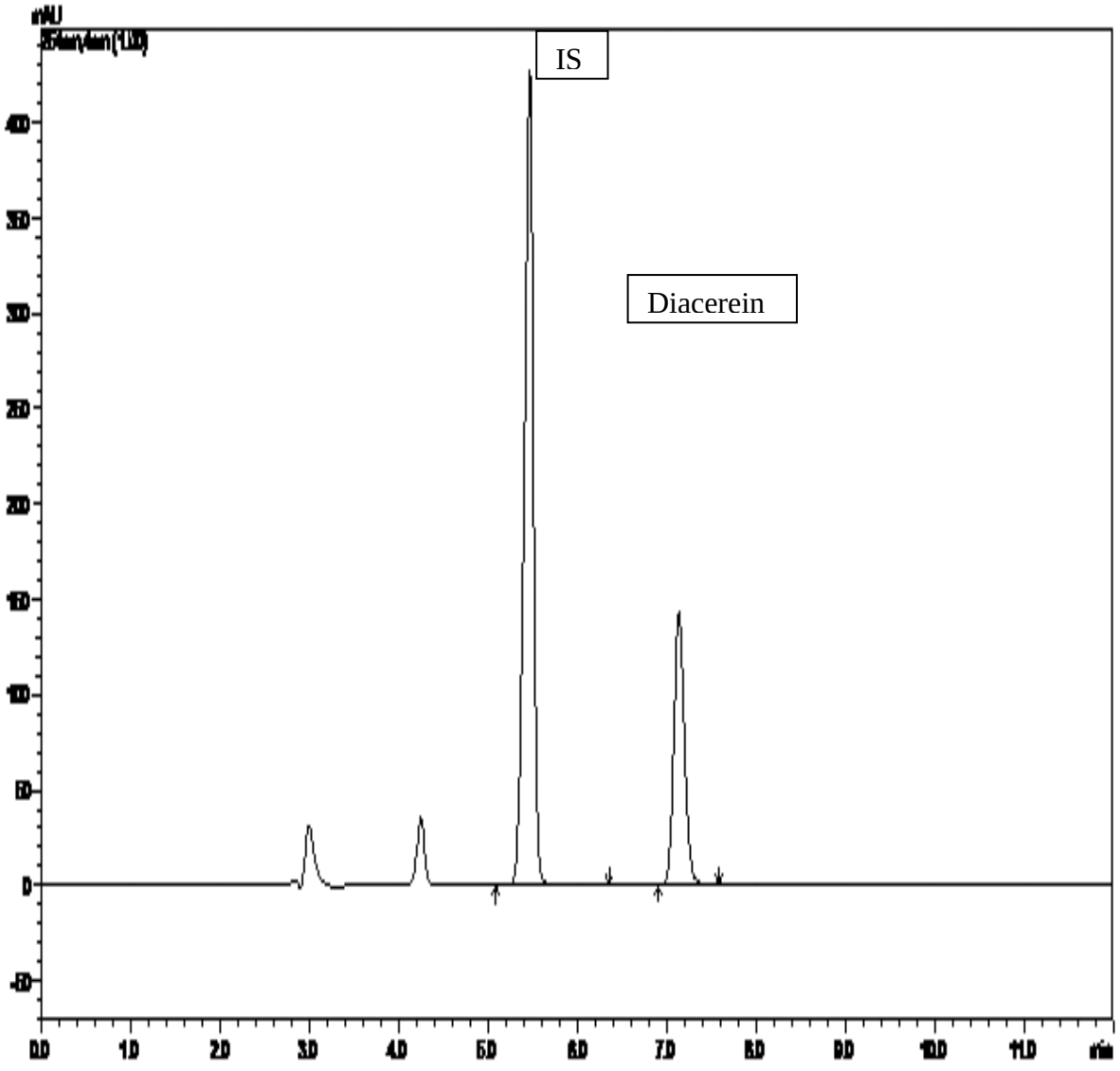


Figure 11.36

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: QC
Sample ID: HQC Freeze Thaw
Injection volume: 20µl
Method File: Calibration

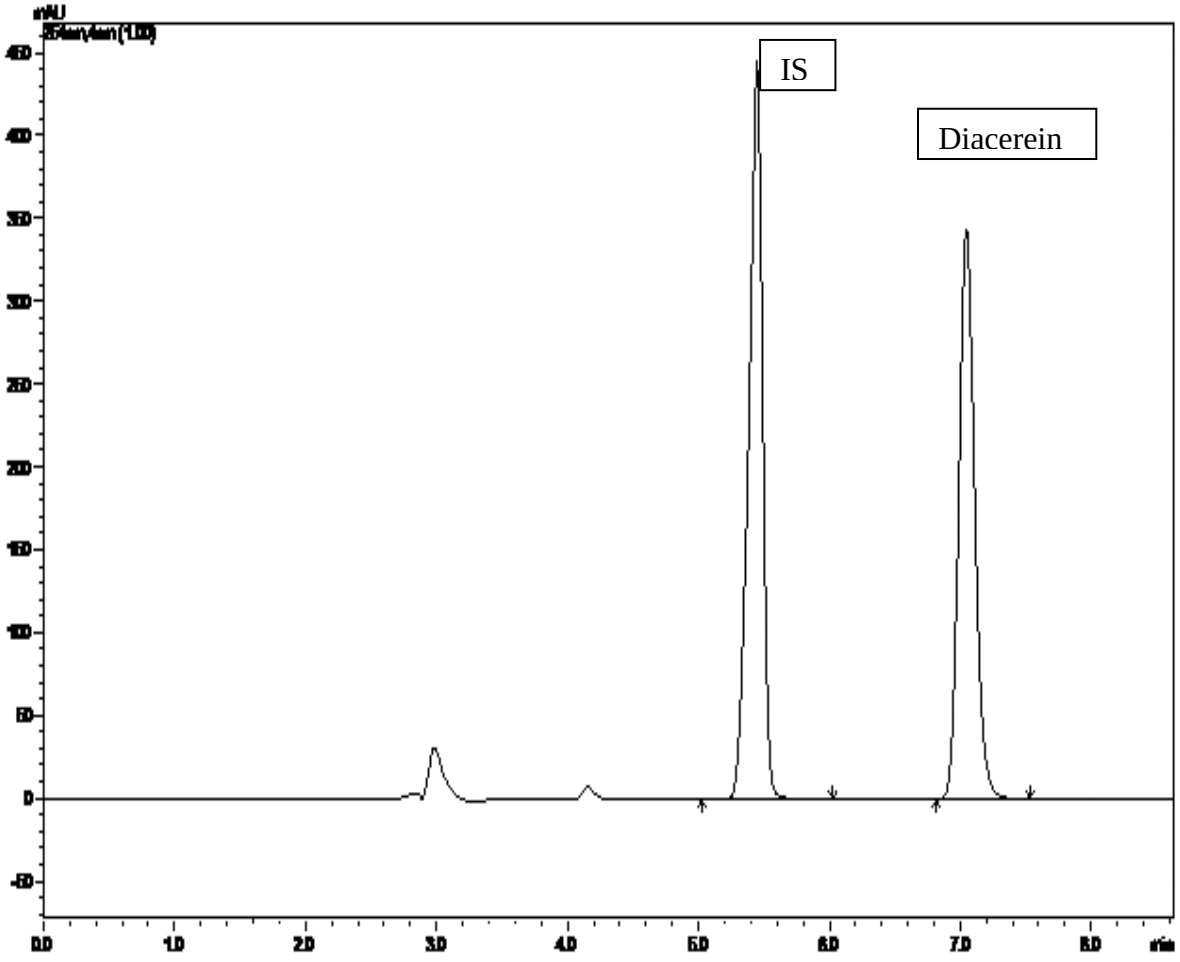


Figure 11.37

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: QC
Sample ID: HQC Freshly Thaw
Injection volume: 20µl
Method File: Calibration

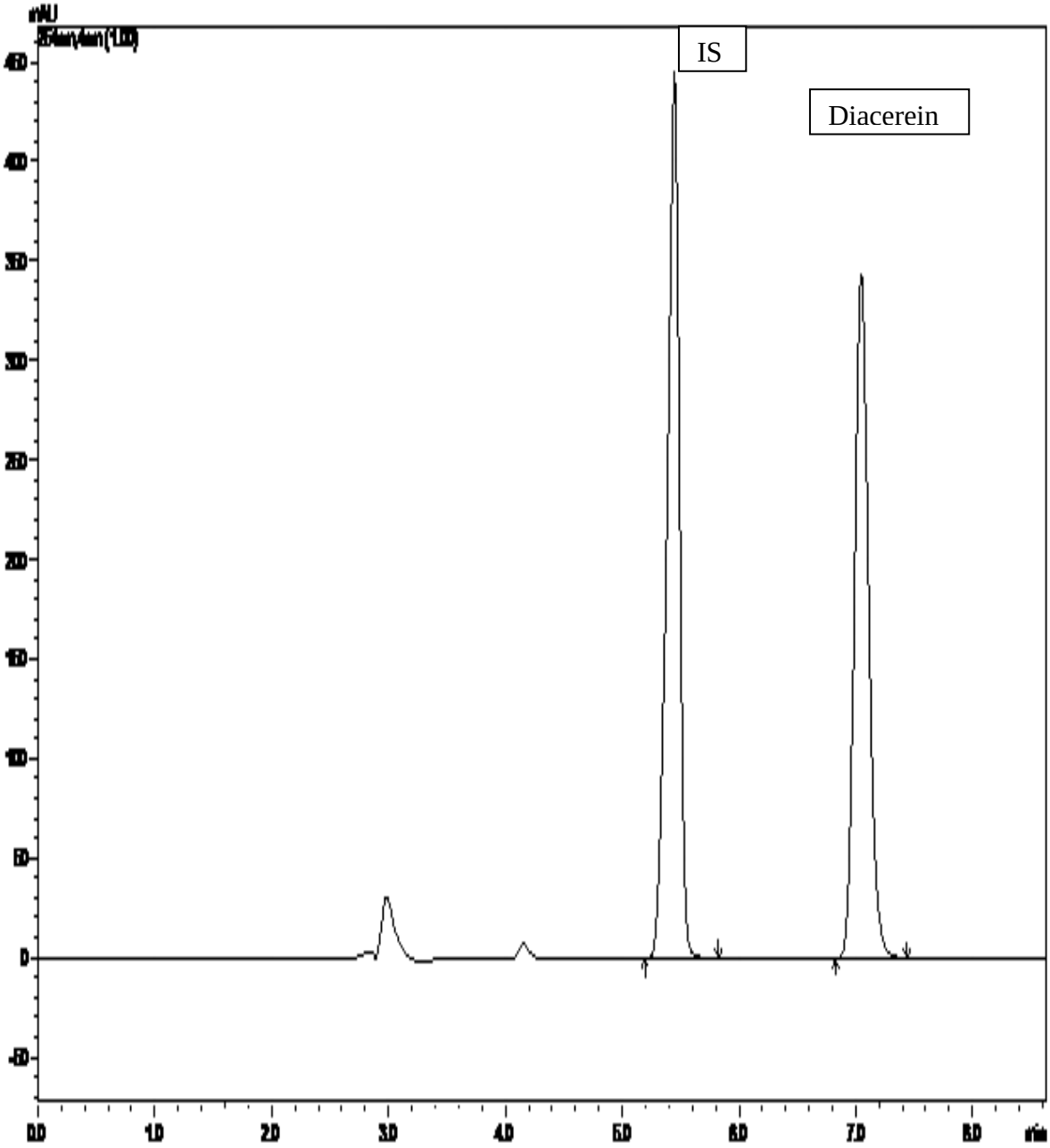


Figure 11.38

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: QC
Sample ID: HQC in Plasma
Injection volume: 20µl
Method File: Calibration

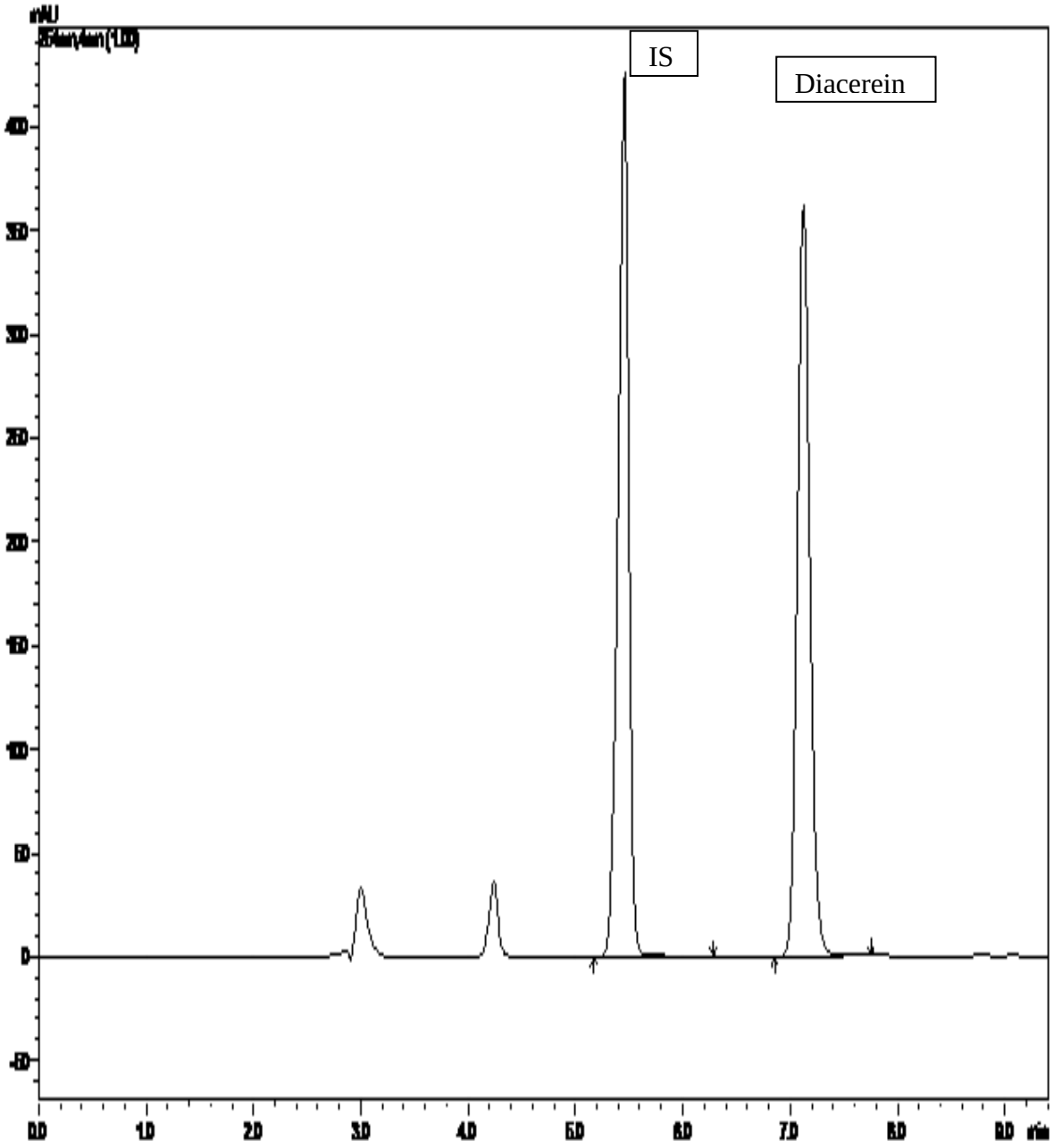


Figure 11.39

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: QC
Sample ID: HQC in Diluent
Injection volume: 20µl
Method File: Calibration

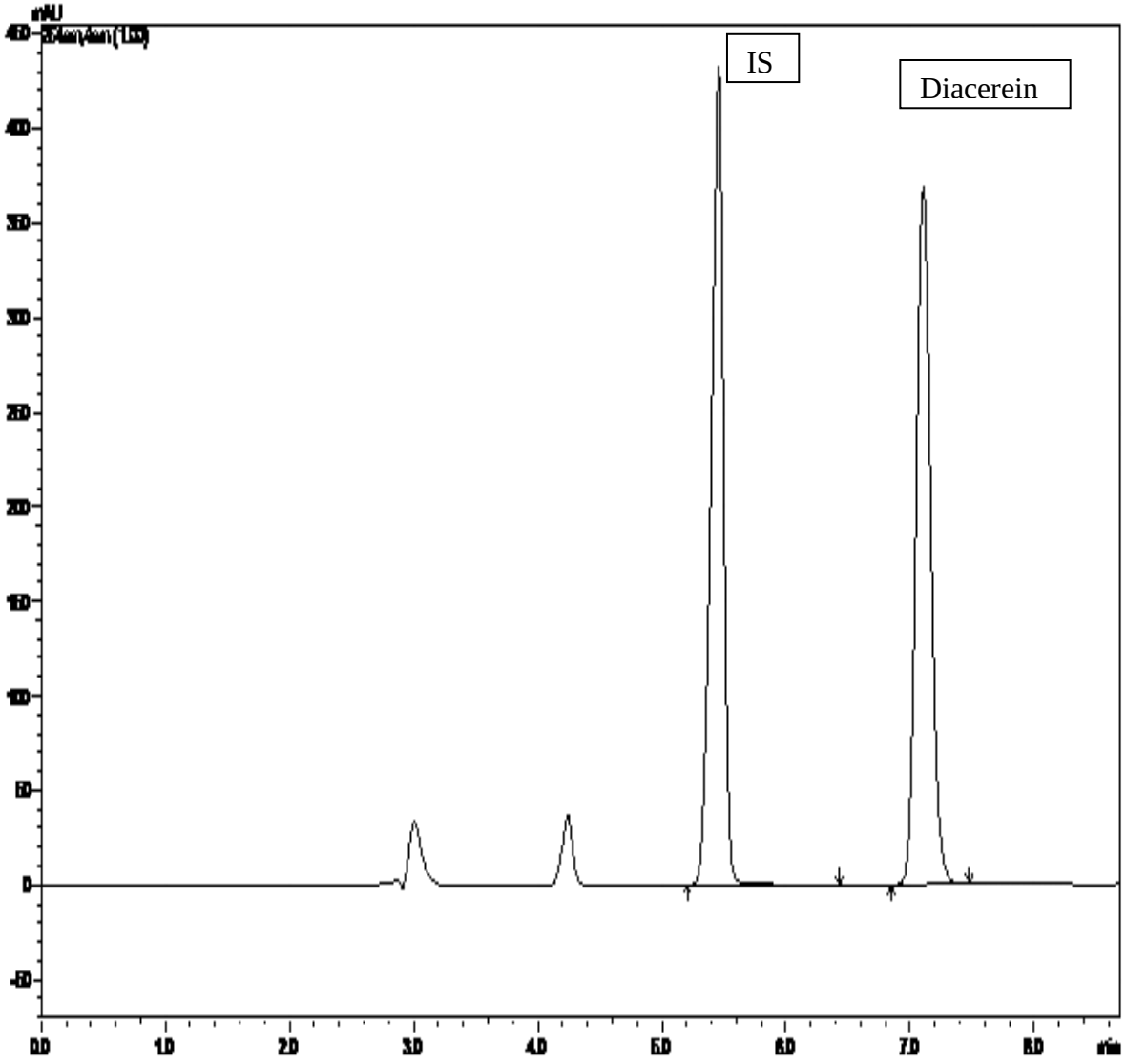


Figure 11.40

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: Rabbits Plasma
Sample ID: 0 Hours
Injection volume: 20µl
Method File: Calibration

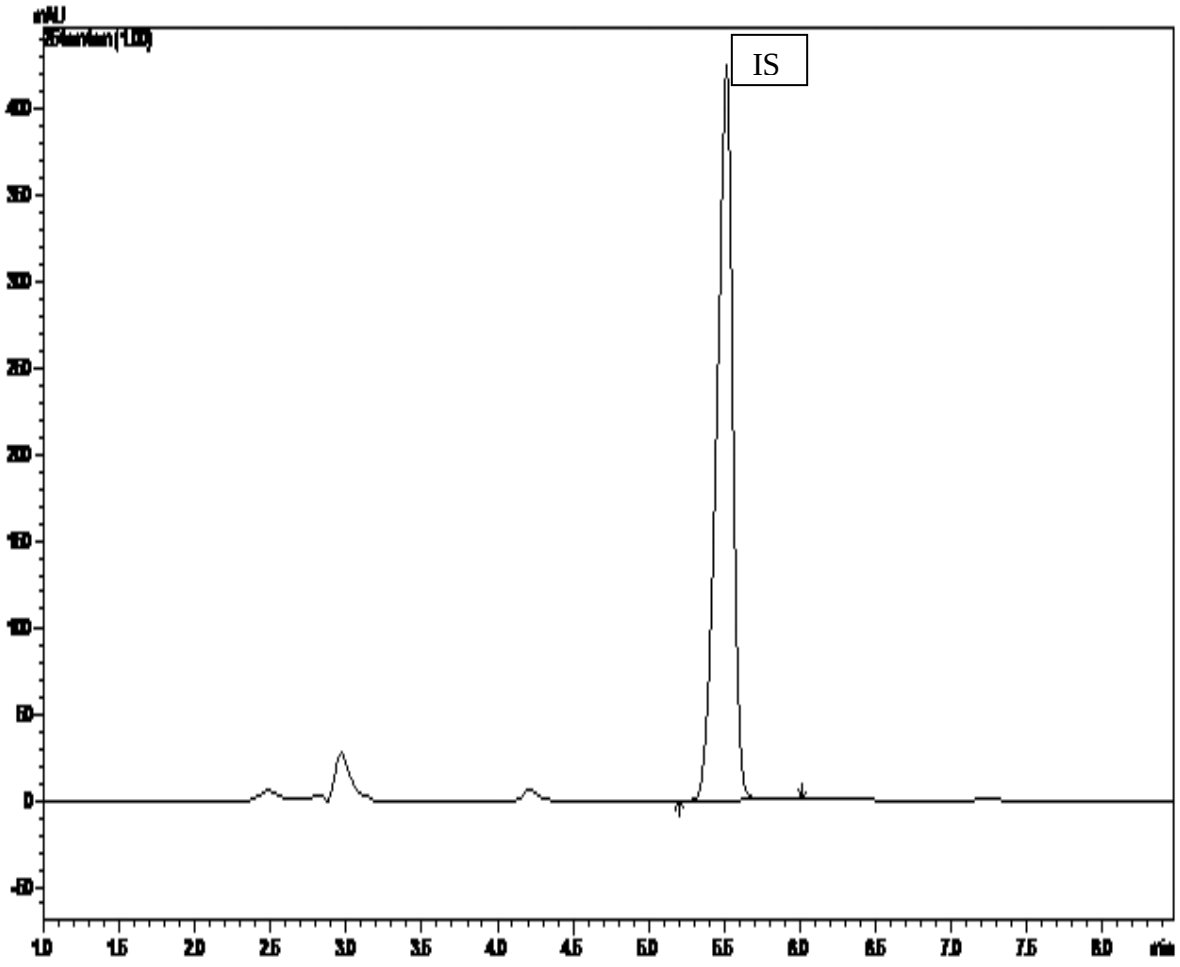


Figure 11.41

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: Rabbits Plasma
Sample ID: 0.5 Hours
Injection volume: 20µl
Method File: Calibration

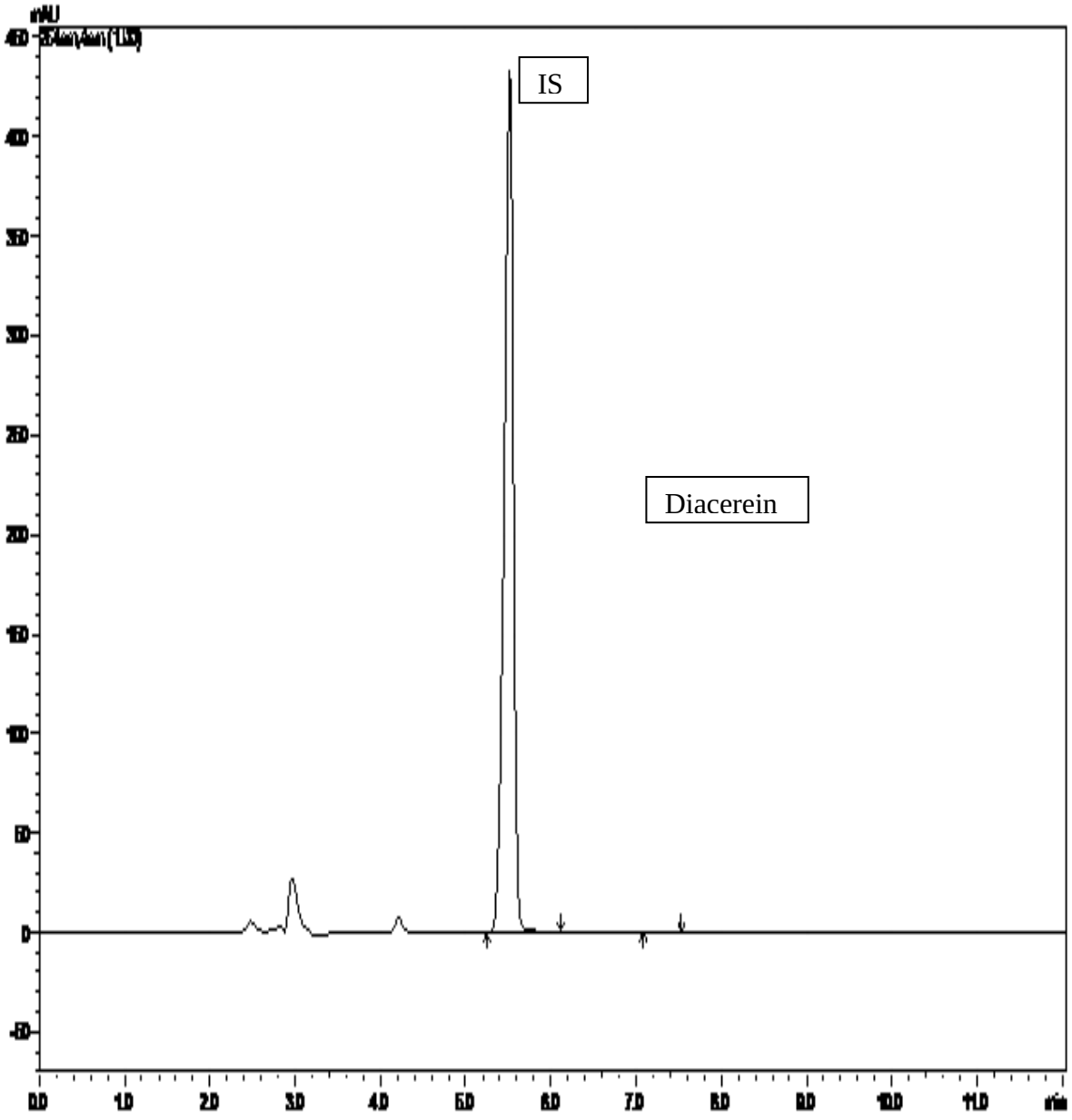


Figure 11.42

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by:	Admin
Sample name:	Rabbits Plasma
Sample ID:	1 Hours
Injection volume:	20µl
Method File:	Calibration

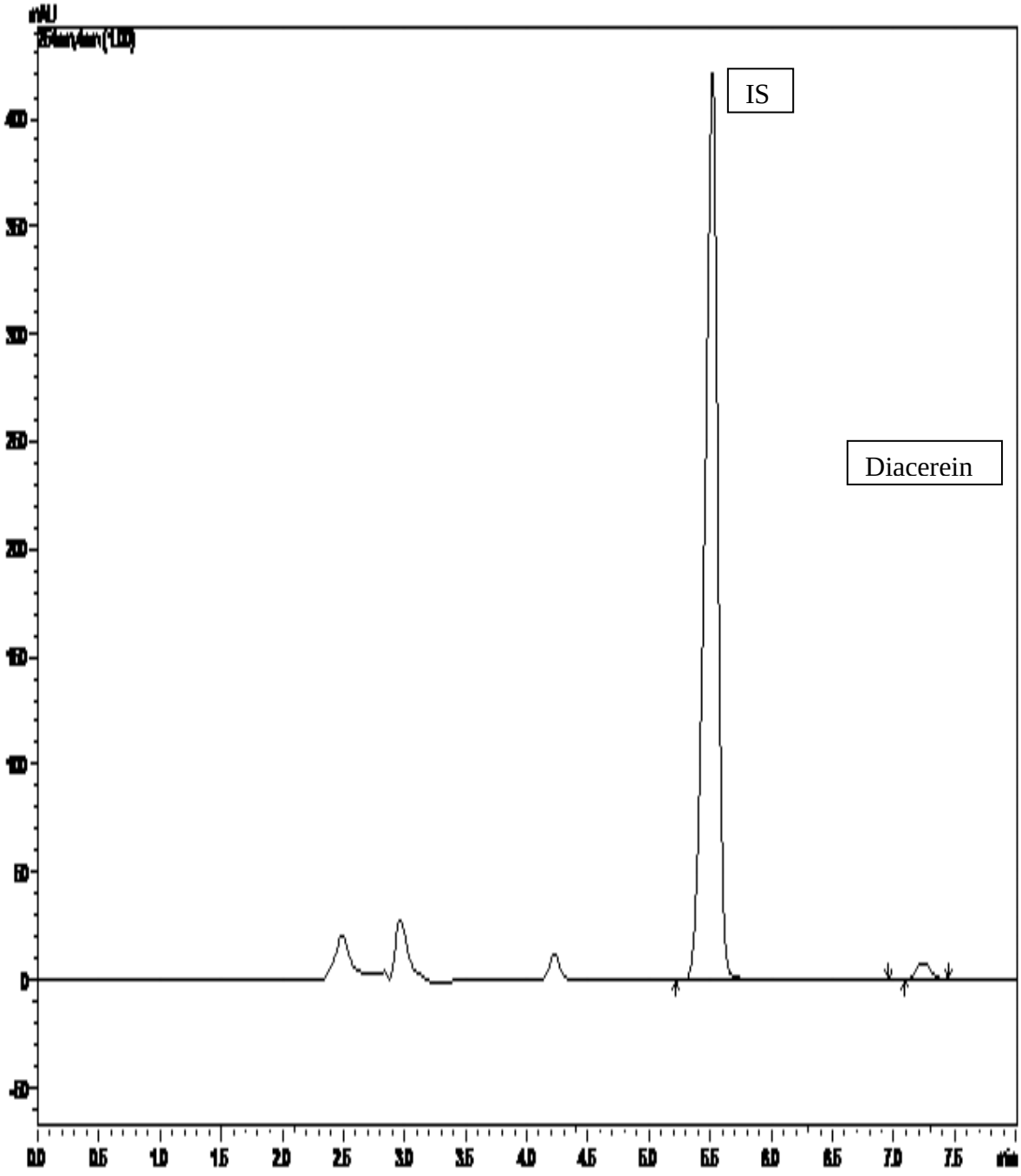


Figure 11.43

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: Rabbits Plasma
Sample ID: 1.5 Hours
Injection volume: 20µl
Method File: Calibration

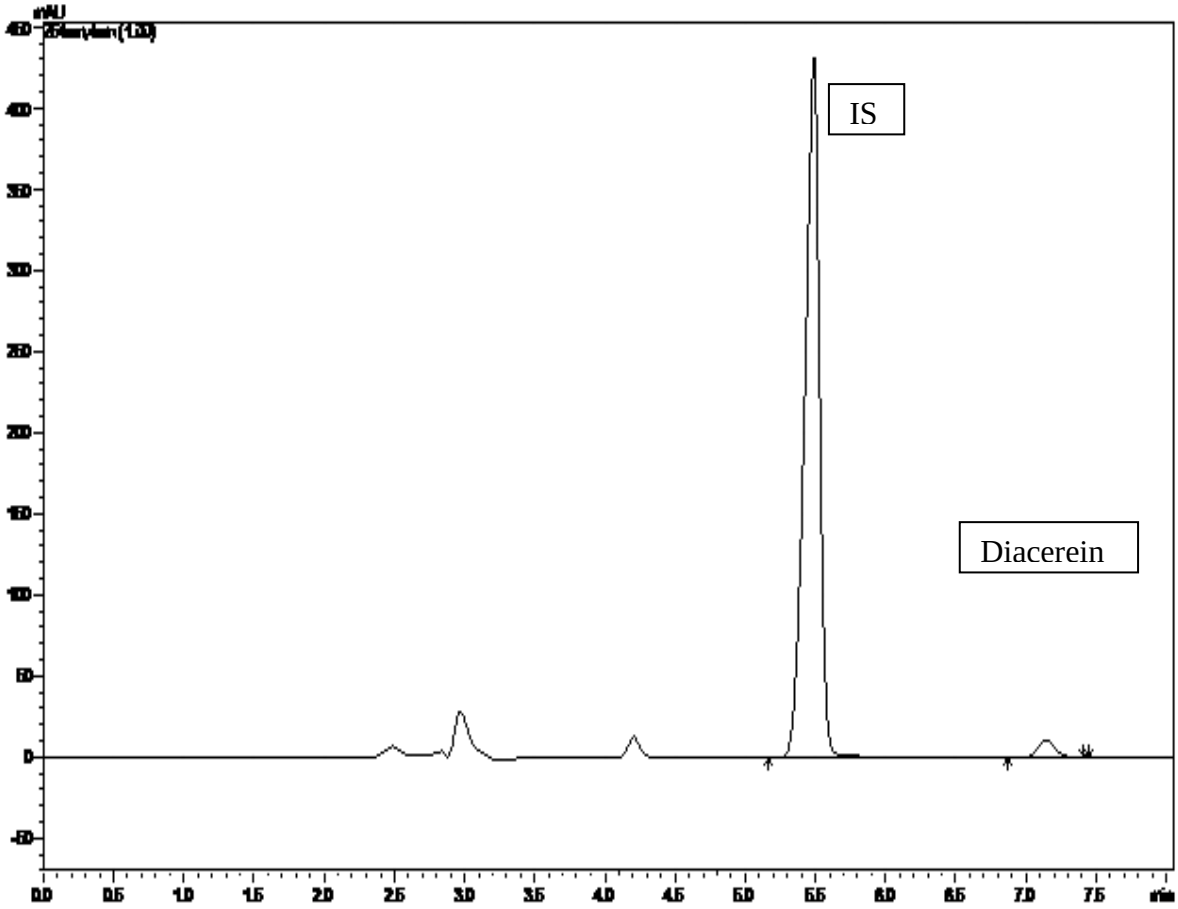


Figure 11.44

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: Rabbits Plasma
Sample ID: 2 Hours
Injection volume: 20µl
Method File: Calibration

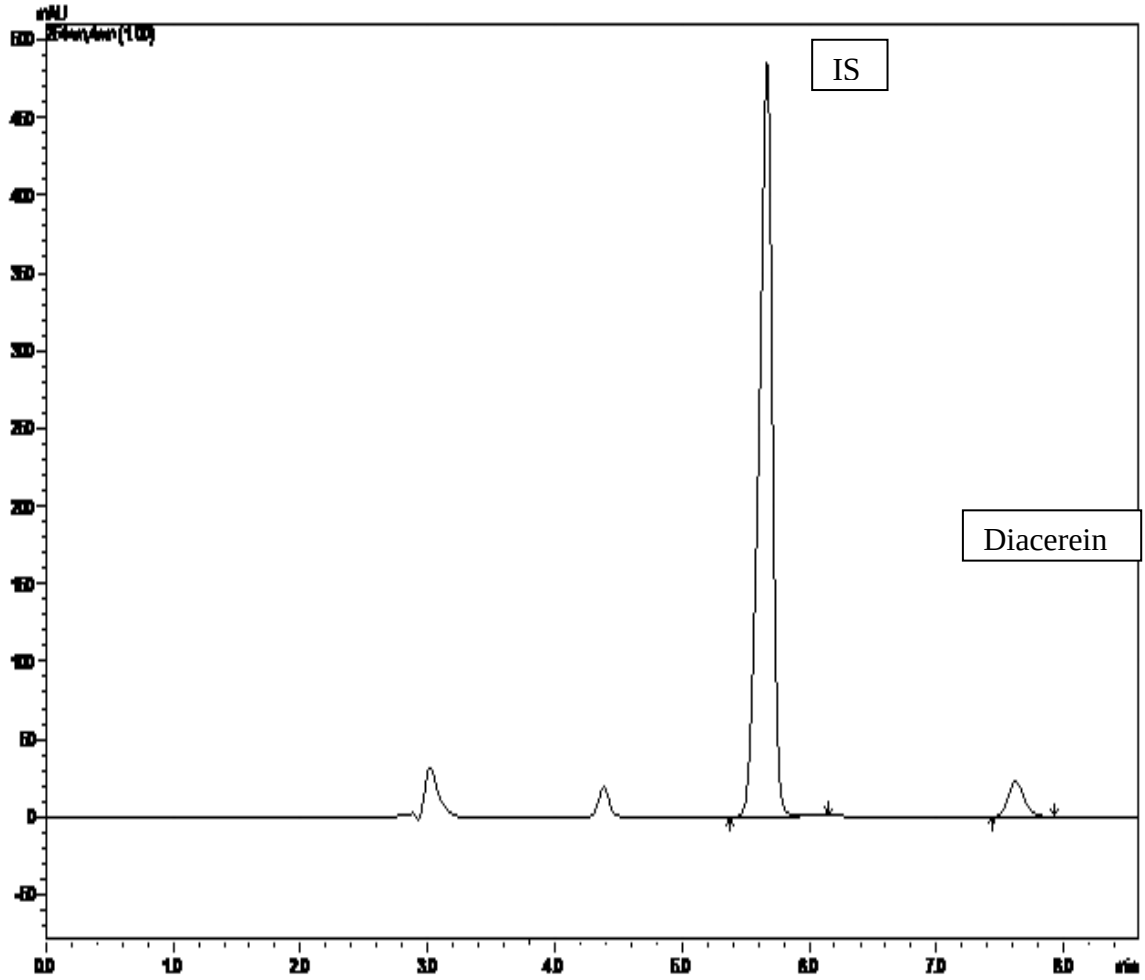


Figure 11.45

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: Rabbits Plasma
Sample ID: 2.5 Hours
Injection volume: 20µl
Method File: Calibration

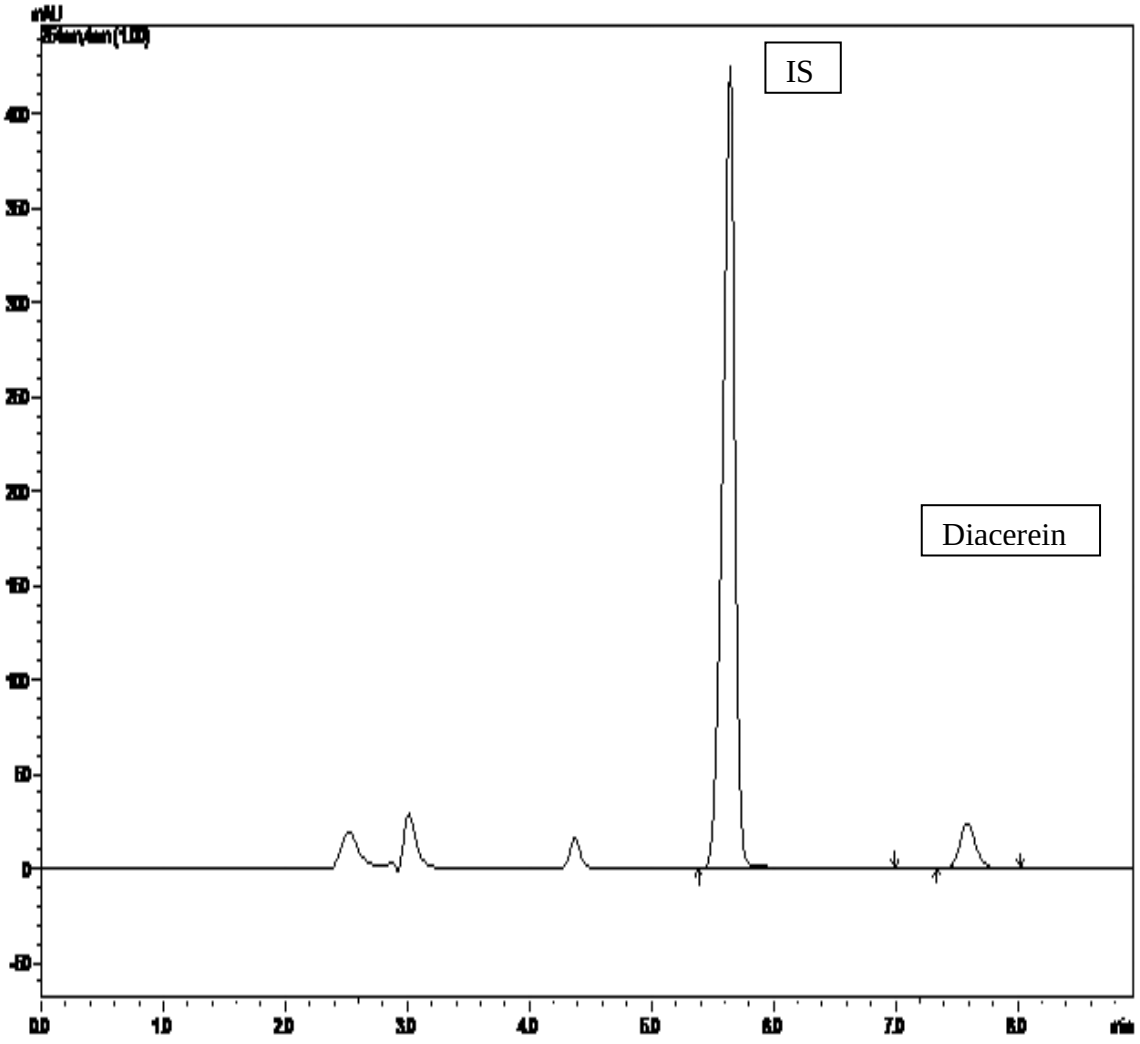


Figure 11.46

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: Rabbits Plasma
Sample ID: 3 Hours
Injection volume: 20µl
Method File: Calibration

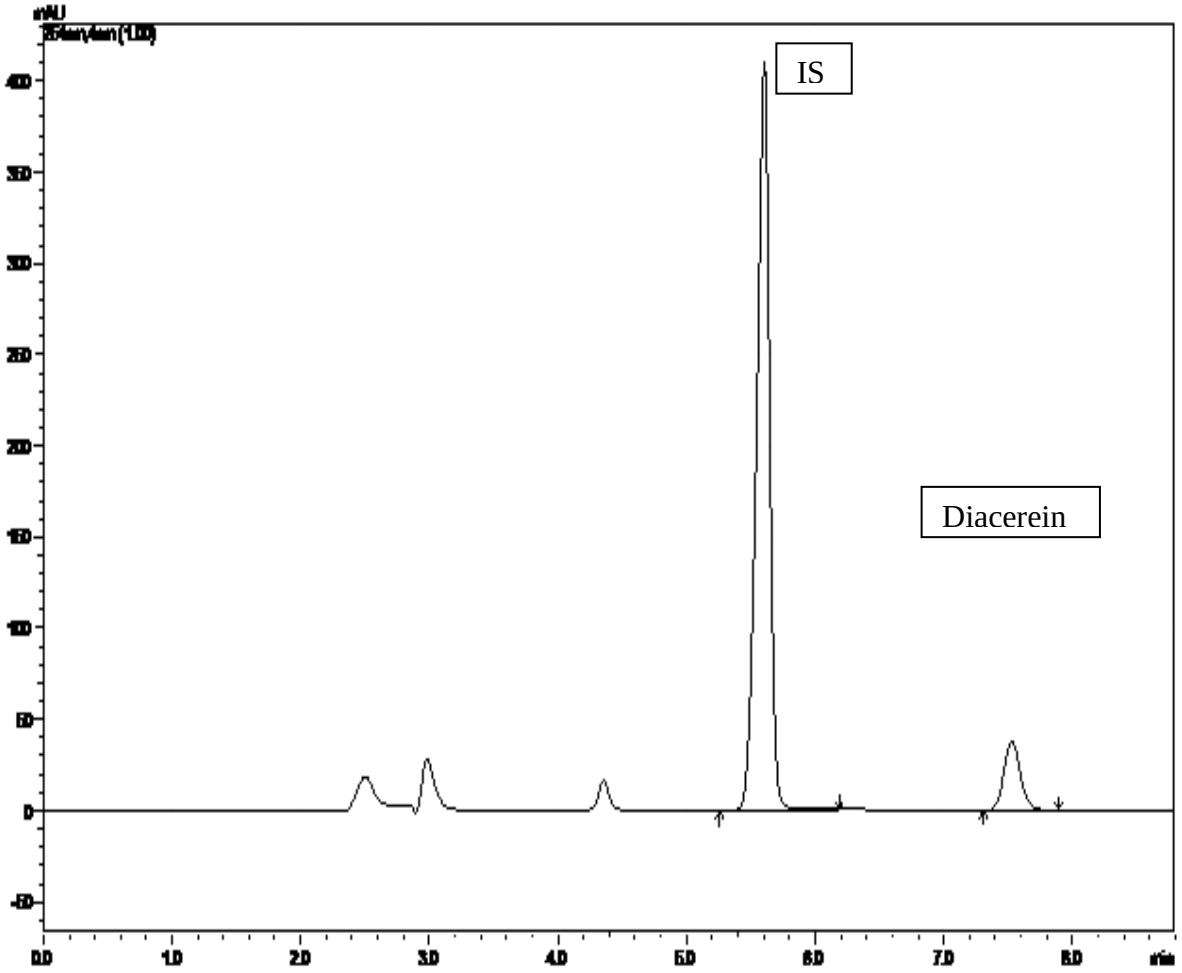


Figure 11.47

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: Rabbits Plasma
Sample ID: 4 Hours
Injection volume: 20µl
Method File: Calibration

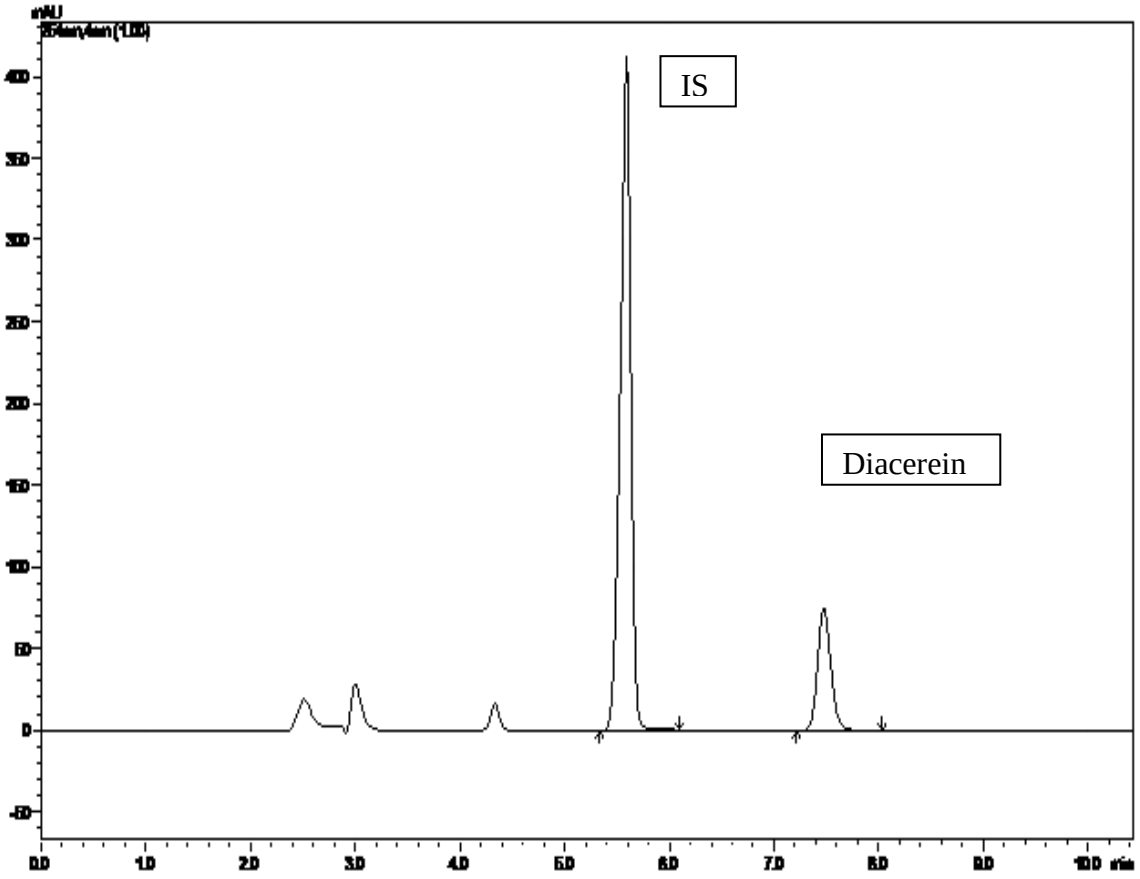


Figure 11.48

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by:	Admin
Sample name:	Rabbits Plasma
Sample ID:	6 Hours
Injection volume:	20µl
Method File:	Calibration

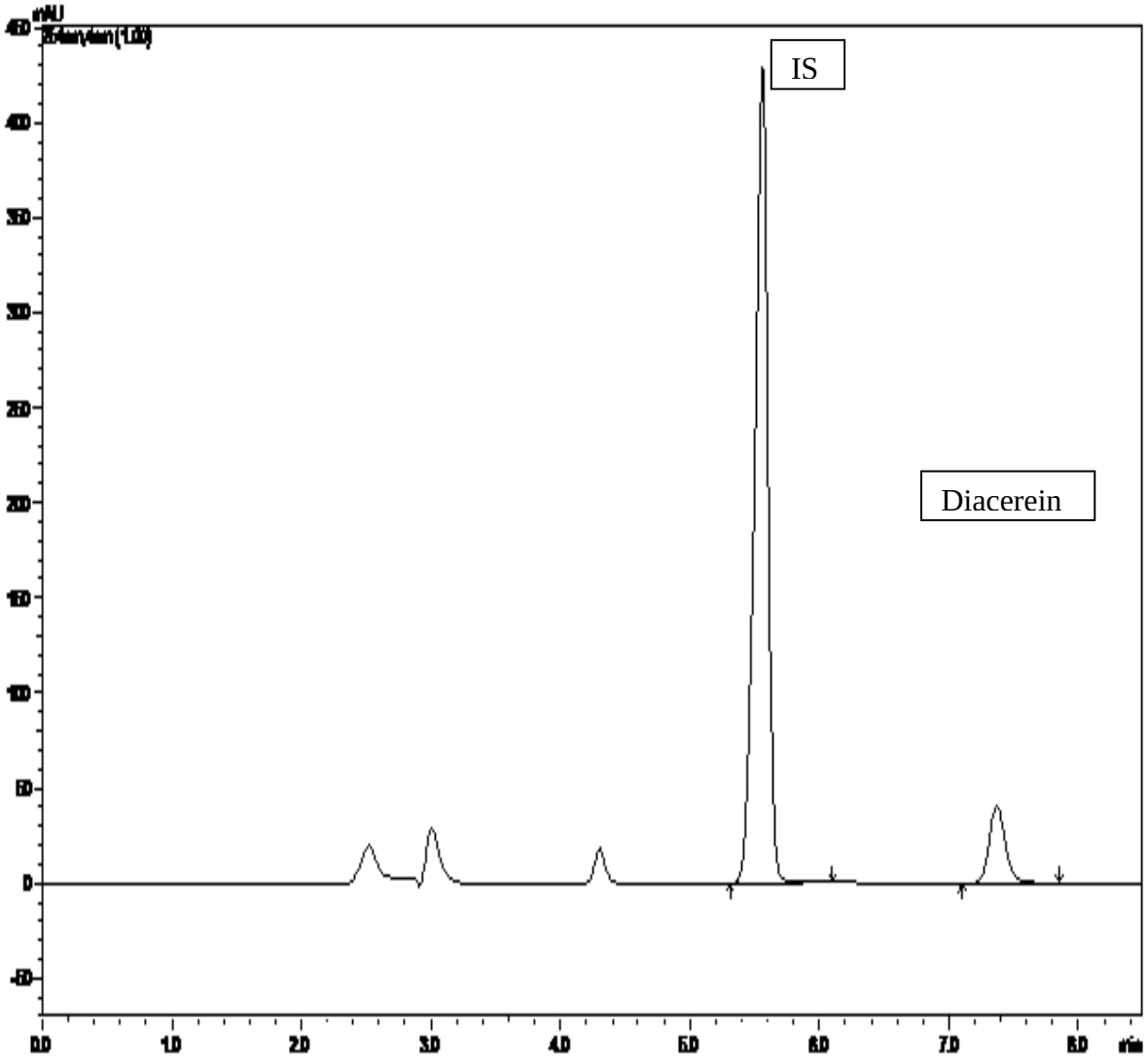


Figure 11.49

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: Rabbits Plasma
Sample ID: 8 Hours
Injection volume: 20µl
Method File: Calibration

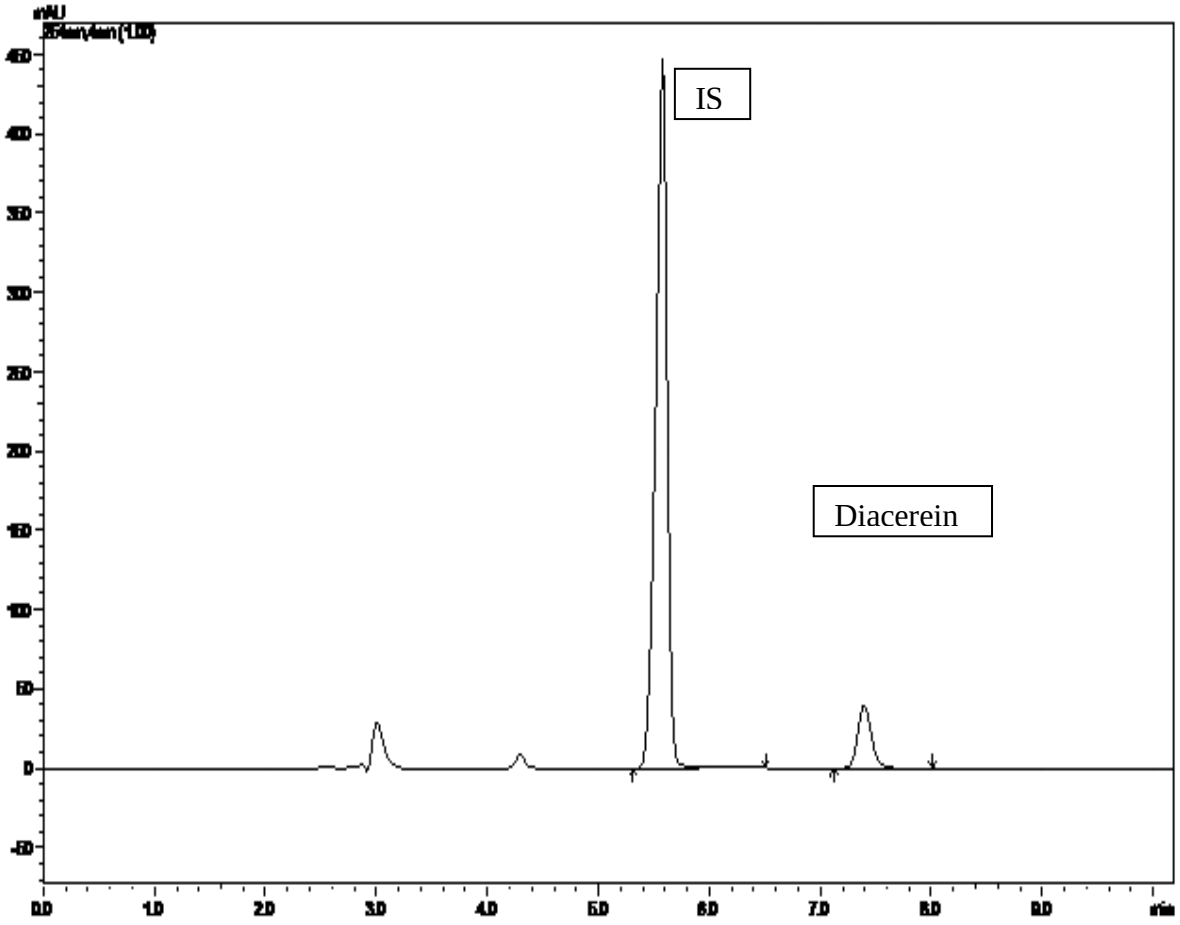


Figure 11.50

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: Rabbits Plasma
Sample ID: 12 Hours
Injection volume: 20µl
Method File: Calibration

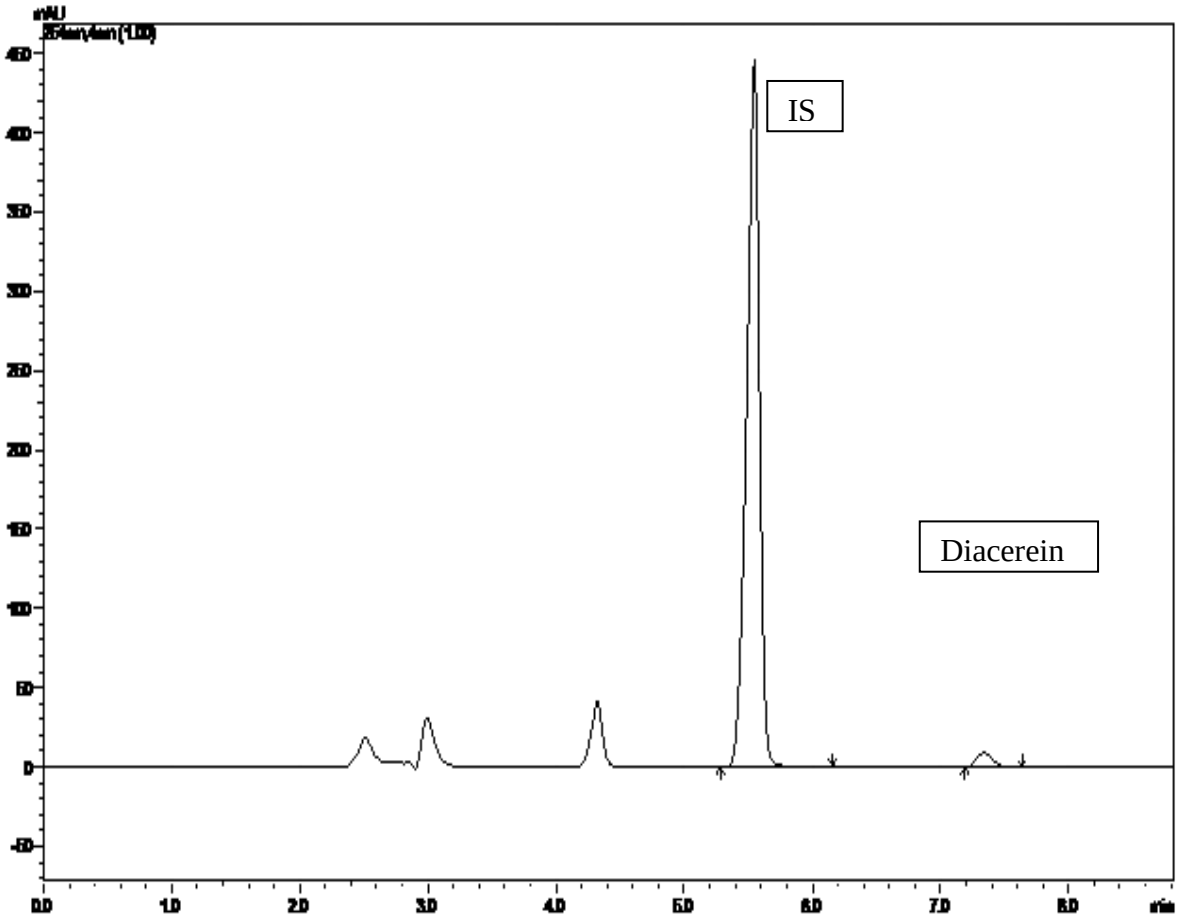


Figure 11.51

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by: Admin
Sample name: Rabbits Plasma
Sample ID: 18 Hours
Injection volume: 20µl
Method File: Calibration

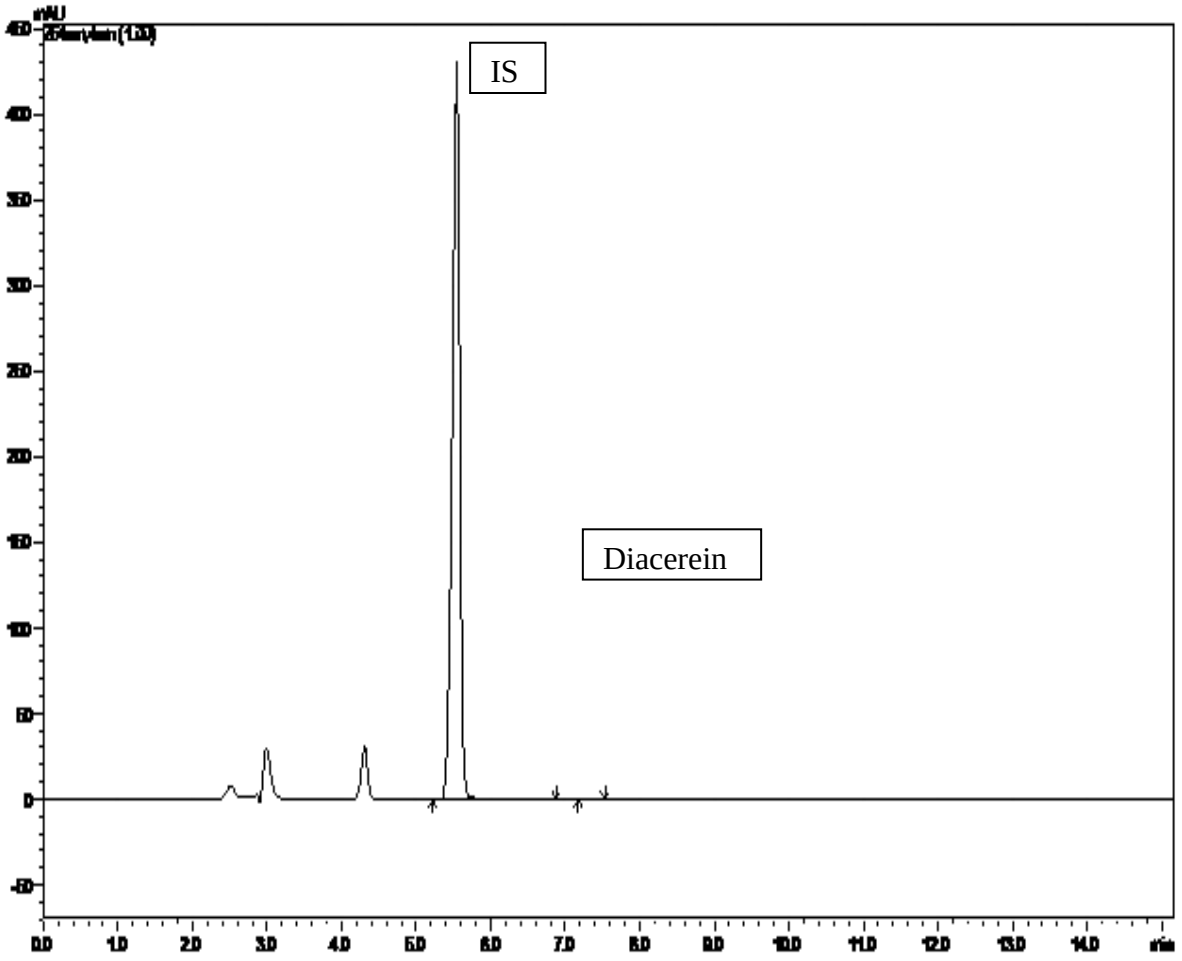


Figure 11.52

D:\Diacerein\Plasma sample analysis\files l.c.d.

Acquired by:	Admin
Sample name:	Rabbits Plasma
Sample ID:	24 Hours
Injection volume:	20µl
Method File:	Calibration

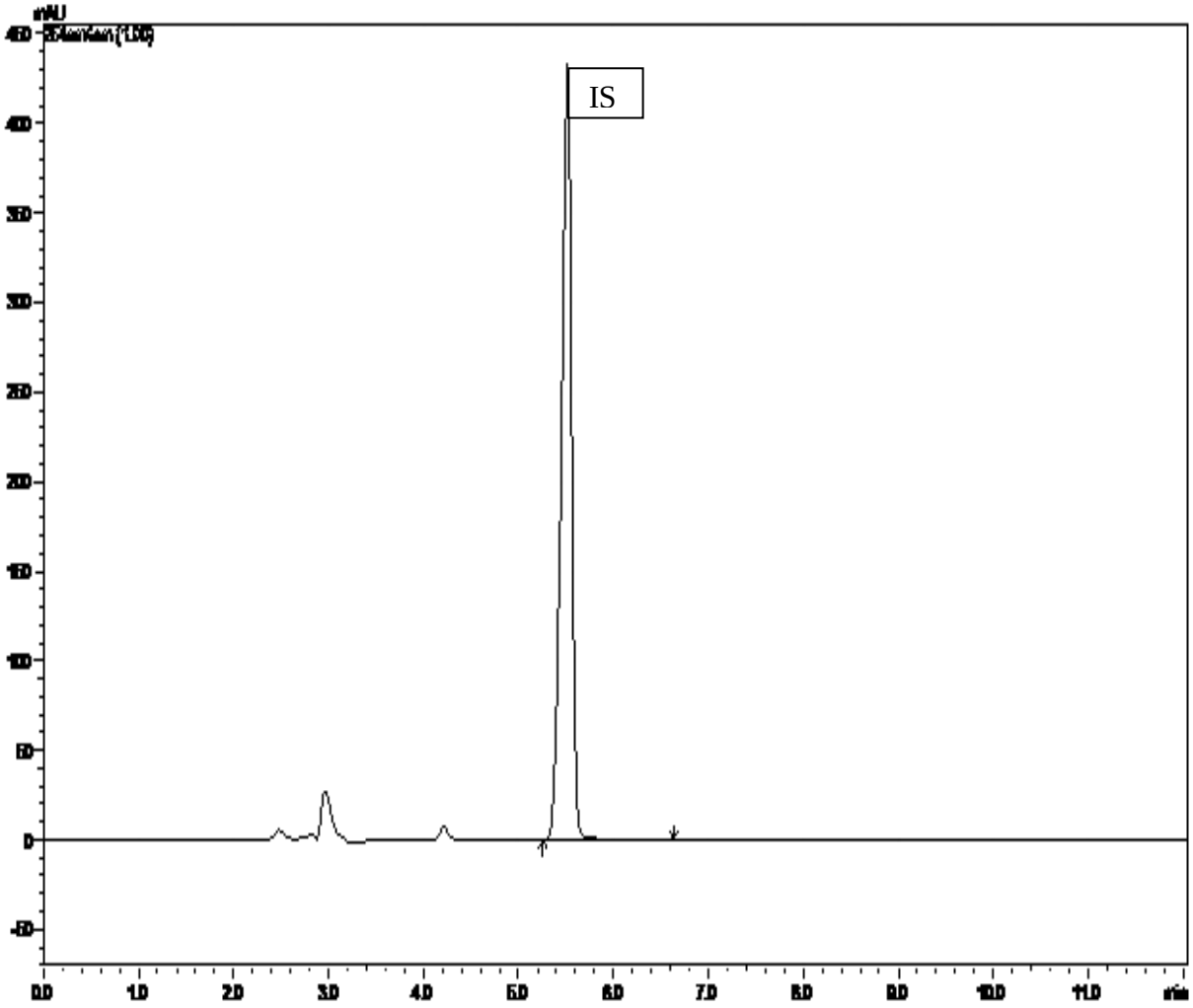


Figure 11.53

CHAPTER-12

RESULTS AND DISCUSSION

Manufacture of microspheres

Microspheres manufactured by Spray coating method are regular, uniform in size compared to other method and batch to batch variation is less. It is easy to scale up bigger batch and can be used for industrial manufacture. It is possible to use instead of organic solvents, aqueous solvent so that problem of residual sovent in the microsphere can be avoided.

Scanning Electron Microscope

DCN Microspheres were prepared in two different processes, one is Double emulsion method and the other is Spray coating method. Polymers used here are Sodium alginate and ethyl cellulose for Double emulsion method and HPMC and Ethyl cellulose for Spray coating method. In case of DCN microspheres prepared by double emulsion method , SEM pictures in different magnifications like 30,60, 100µm showed three dimensional structures as round, porous and non aggregating structure.

In case of Spray coating method size of microspheres came approximately 20- 100µm in size magnification of 144,146, 261, 408, slightly porous, spherical and aggregation free morphology.

Morphology of these two different types of DCN microspheres predicts easy penetration by solvent attack, causes swelling of internal matrix and release of drug by burst of microspheres or drug release by diffusion.

DLS Study (Particle size determination)

DLS study of Diacerein microsphere showed that microspheres are having size between 396.1nm to 615.1nm. Mean percent intensity was found 396.1nm(16%), 458.7nm(33%),531.2nm(33.8%),615.1nm(17%). Hence particle size were having even size distribution and as per definition of microsphere. Zeta potential was between -10.8 to -19.7mv and PDI 0.650, which further showed that the result quality was good.

FT-IR characterization:-

Characterization by FT-IR method of DCN pure drug, DCN microsphere and Drug- polymer and other excipients mixture showed nearly identical spectra in C-H stretch aliphatic, C-H stretch aromatic, C=O ester, C=O stretch COOH, C-O ester, M- substituted benzene,

benzene. Which shows that during manufacture of Diacerein microsphere no structural change takes place. This confirms drug stability and compatibility in the process.

X-RAY Diffraction Spectroscopy (XRD)

Stability and compatibility of drug and polymer with other formulation excipients were studied by XRD spectroscopy. Comparison of XRD patterns of DCN pure drug, DCN microsphere and Drug polymer and other ingredients physical mixture showed three different diffraction pattern. It is known that each diffraction pattern is characteristic to a specific crystalline structure of a sample. XRD can fingerprint what is the composition of a sample, it can also predict the crystalline phase in a sample. In a sample mixture it determines the sum of diffraction pattern of individual phase.

Study showed that there was little shift in crystalline structure of the pure drug, formulation and physical mixture. Slight shift in crystalline structure in microsphere and physical mixture might be due to lowering of the purity of drug.

Differential Scanning Calorimetry (DSC)

Comparative DSC analysis of DCN as pure drug, microsphere and physical mixture depicted three endothermic peak close to each other. Shift in peak in case of DCN microsphere and Physical mixture may be due to the presence of polymer and other excipients. Thus it indicates the absence of chemical interaction between drug, polymer and excipients.

The DSC study was carried out in dynamic nitrogen atmosphere with a constant flow of 30ml/min, and heating rate of 10^{0c}/min up to temperature of 300^{0c}. The sample with a mass of 1.8mg was packed in platinum pan . DSC equipment was primarily calibrated with standard reference

In – vitro release study:-

Dissolution rate study of Diacerein microsphere and conventional capsule available in the market were carried out in different pH medium like phosphate buffer (ph 6.8), citrate buffer(6.0) and in acidic medium (0.1N HCl) to understand the drug release pattern. Release of drug from a dosage form Theoretically involves both diffusion and dissolution and several mathematical equation or model can describe dissolution and /or release from drug delivery system. Release study showed that conventional Diacerein capsule released its 80 %drug content in one hour in both phosphate buffer (ph 6.8) and in citrate buffer (ph 6.0) and in

0.1N Hcl drug release was neglible. Release rate of DCN from microspheres showed sustained or delayed release in comparison with conventional marketed DCN capsule. This sustained or delayed release was observed in both above mentioned buffers. No or neglible Drug release was found in acidic medium (0.1N HCl).

Drug release from microsphere was found to be 97.6% in 20 hr in case of phosphate buffer at pH 6.8. In case of citrate buffer drug release was found to be about 60% in 24 hr. Which proved doubtlessly that microspheres showed sustained release action.

In- Vitro drug release kinetics :-

Objective of the present study was to formulate Diacerein microsphere as sustained release dosage form and its release profile was interpreted with various mathematical models. DCN microsphere's dissolution rate in phosphate buffer (pH 6.8) was studied in Zero, first, Higuchi, Korrsmeyer and Hicxon- Crowell mathematical model

Out of which based on highest value of correlation coefficient (r^2 = 0.988),in- vitro drug release kinetics showed that Higuchi square root model was best fit for release of drug from phosphate buffer pH 6.8.

Thus the plot of cumulative % drug release vs square root of time as per graphical representation in fig 20 showed the Higuchi plot. It can be interpreted that prime mechanism of drug release is by diffusion. However, it may be initially swelling of polymer matrix and thereafter release of drug by diffusion.

In-vivo study

Comparative bioavailability study of Diacerein microsphere with conventional Diacerein capsule (marketed prep.) in rabbit plasma showed that peak plasma concentration (Cmax) of microsphere was 4µm in both the formulations. But time to reach Cmax was 2.6hr in conventional capsule whereas in case of microsphere it was 4hr. In case of microsphere drug was found in the plasma up to 18 hr whereas in case of capsule it was up to 10hrs. There were increase in AUC0-t and AUC0-∞ in case of microsphere and also increase in elimination rate constant. However, biological half life(t1/2) was less in case of microsphere. which are all supportive of sustained release drug delivery in case of microsphere formulation.

CONCLUSION

Aim of the current research work was to develop and optimize sustained release drug delivery of Diacerein as microsphere formulation by using Double emulsion and Spray coating (wurster method) using low cost, easily available, nontoxic, inert polymer.

Diacerein microspheres were characterized to see its drug entrapment, assay, stability and compatibility of drug and polymer and excipients. Dissolution rate (In- vitro) study was done to confirm sustained/ delayed release action. The pharmacokinetic parameter study of microsphere was done in rabbit plasma and compared with conventional capsule available in the market. Next, an attempt was made to establish correlation between in-vitro and in- vivo data (IVIVC) between in- vitro drug release and in vivo absorption of Diacerein from the sustained release microsphere formulation.

1) Optimization of process parameter of Diacerein microsphere :

Microspheres formulated by spray coating or fluid bed bottom coating (wurster method) was found uniform spherical and free flowing. Advantage of adopting spray coating for manufacture of Diacerein microsphere is a completely new concept where quantity of organic solvent used was small and drying at low temperature and easy to scale up for commercial manufacture. Whereas Double emulsion method could be used for research purpose only as drug entrapment was low and commercially not viable.

Scanning Electron Microscopy study was used to check the surface topography. Spherical porous surface ensured uniform drug release from the microsphere. Drug content in microsphere was assayed by HPLC method. Method was validated with the method with the method described in IP2014 monograph of Diacerein capsule. Drug loading and entrapment efficiency of the microsphere was calculated . The result revealed that the microsphere had good drug entrapment capacity (more than 60%) by Spray coating process.

Particle size, size distribution, zeta potential and poly dispersity index of microsphere showed uniform distribution pattern. Zeta potential and PDI showed that microsphere was stable with good result quality.

2) Compatibility study of Diacerein microsphere :

Interactions of drug-polymer and drug excipients in microsphere were confirmed by FTIR, XRD and DSC study. These studies showed that no structural change or incompatability took place during formulation.

3) Dissolution rate (in-vitro) study from Diacerein microsphere :

Dissolution rate (in- vitro) was studied in different buffers and acidic pH. It could be viewed easily that drug release from microsphere was extended up to 80%in 24 hr in case of phosphate buffer pH 6.8 and in case of citrate buffer pH 6.0, up to 60% in 24hrs. Whereas Diacerein conventional capsule released its drug content 80% in 1hr. This has justified the concept of Diacerein microsphere as sustained/ delayed release formulation as per aims and objectives of this thesis.

4) Release kinetics study of Diacerein microsphere :

Different mathematical equations are used to explain the in- vitro drug release character of S.R. DCN microspheres. Out of two different methods followed here for preparatioo of DCN microsphere namely Double emulsion and Spray coating , based on the linearity of regression co-efficient, r^2 value, microsphere of double emulsion method followed Koresmeyear & Peppas model (1983) and Spray coating method followed Higuchi model (1962). This explains that drug release form microspheres prepared by double emulsion method followed bursy release method and in case of microspheres prepared by Spray coating followed Diffusion kinetics.

Comparative in-vivo drug release study in rabbit plasma of Diacerein microsphere and Diacerein capsule (marketed prepn.) showed that

Formulation	Microsphere	Conventional Capsule
C_{max}	4.02 µg	3.97µ g
T_{max}	4.0 hr	2.67 hr
AU C_{0-t} mcg. hr/ml	21.26	17.80
$AUC_{0-\infty}$ mcg. hr/ml	21.35	19.38
K_{el}	0.35	0.31
$T_{½}$	1.98	2.30
C_{min}	0.031 µg in 18hrs	0.046 µg in 10 hrs

The above findings clearly showed that there were increase in Cmax (1.25 %), Tmax (49.8%), AUC $_{0-t}$ (19.4%), $AUC_{0-\infty}$ (10%), Kel (12.9%), $T_{1/2}$ (-13.91%). Enhancement of Tmax and AUC of microsphere showed clearly that developed microsphere formulation had sustained release drug delivery over conventional capsule formulation available in the market. Biological half life of microsphere was about 1.8hr and in case of capsule it was about 2.30hr.The lowering of half life in case of microsphere compared to conventional capsule could not be properly explained. However, it can be concluded without any doubt that the study meets its objectives. Further, in vivo study on comparatively large laboratory animal species like dog and if the study can be carried out on human volunteers it will prove conclusively that the developed formulation has sustained release drug delivery

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Preparation And Evaluation Of Diacerein Loaded Microsphere With Sodium Alginate and Ethyl cellulose by Double Emulsion Solvent Evaporation Method' Int j Pharm Bio Sci; 7(3) : (P) 231-239(6)

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ANNXURE 1

International Journal of Pharma and Bio Sciences 0975-6299

PREPARATION AND EVALUATION OF DIACEREIN LOADED MICROSPHERE WITH SODIUM ALGINATE AND ETHYL CELLULOSE BY DOUBLE EMULSION SOLVENT EVAPORATION METHOD

SOUMEN MUKHOPADHYAY, SHARMILY CHAKRABORTY, SUMANTA DAS, AND Dr.TAPAN KUMAR CHATTERJEE*

Division of Pharmacology, Department of Pharmaceutical Technology, Jadavpur University, Kolkata-32.

ABSTRACT

The present study was conducted to prepare microsphere of poorly water soluble drug Diacerein by double emulsion method (W/O/W) where sodium alginate was used as drug release retarding polymer in microsphere formulation and ethyl cellulose was used as coating polymer. Diacerein was found to be compatible with matrix polymer and co-polymer by conducting the various physicochemical and instrumental analyses. Three different Diacerein loaded microspheric formulations were prepared using variable concentrations of Sodium alginate and Ethyl cellulose as it was found that entrapment efficiency and particle size of the microspheres were increased on increasing polymer concentration of formulations and on increasing polymer concentration the drug release of all the formulations were gradually decreased.Though not being categorized as anonsteroidal anti-inflammatory drug, Diacerein has significant role in pain therapy. It can be concluded that preparing Diacerein microsphere using sodium alginate and ethyl cellulose polymers can be a new addition in the field of pain management as sustained release therapy.

KEYWORDS: Microsphere, Diacerein, Sodium alginate, Ethyl cellulose

Dr. TAPAN KUMAR CHATTERJEE
Division of Pharmacology, Department of Pharmaceutical Technology, Jadavpur University, Kolkata-32.

ANNXURE -2

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Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Formulation Development and Evaluation of Diacerein Loaded Microsphere by Spray Coating (Wurster Method)

Soumen Mukhopadhyay[1], Tathagata Roy[2] & Tapan Kumar Chatterjee*[3]

[1] Division of pharmacology, Department of pharmaceutical Technology, Jadavpur University, Kolkata 700032

[2,3] Depertment of pharmaceutical technology, JIS University, Kolkata 700109

ABSTRACT

Diacerein loaded microspheres were prepared by spray coating(wurster method) using hydroxyl propyl methyl cellulose (HPMC) and ethyl cellulose as release retarding polymer with a view to manufacture sustained release drug delivery. Drug content in the microspheres was determined by HPLC assay followed by drug entrapment efficiency. Shape and Surface topography of Diacerein loaded microspheres was determined by scanning electron microscopy. Fourier transform infrared spectroscopy (.FT-IR), X-ray diffraction Spectroscopy (XRD), and Differential scanning calorimetry (DSC) studies were done to establish drug polymer and other excipients compatibility and stability. Sustained release action was established by In-vitro release study. The result shows that Diacerein loaded microsphere using hydroxyl propyl methyl cellulose and ethyl cellulose polymer can be a new addition in the field of pain management for the treatment of osteoarthritis.

Keywords: Microsphere, Diacerein, HPMC, Ethyl cellulose, Spray coating.

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***Address for Correspondence:**

Prof (Dr.) Tapan Kumar Chatterjee, Department of Pharmaceutical Technology, JIS University, Kolkata-700109

INTRODUCTION

Nowadays conventional dosage forms of drugs are rapidly being replaced by the new and novel drug delivery systems, among these sustained release or control release dosage forms are very popular in present day therapy. Diacerein or Diacetylrhein comes under the class anthraquinone derivative. Chemically it is 9,10-dihydro-4,5-dihydroxy-9,10-dioxo-2-anthranoic acid diacetate[1]. Diacerein is thought to act via inhibition of interleukin-1Beta, a protein involved in the inflammation and destruction of cartilage that play a role in the development of symptoms of degenerative disease like osteoarthritis. Diacerein is a short acting drug, practically insoluble in water. Oral bioavailability of Diacerein is about 35to56%. Hence, the drug was selected for preparation of sustained release formulation. The study design was to prepare microsphere of Diacerein by spray coating with HPMC and ethyl cellulose as coating polymer which not only provides prolong therapeutic action but also reduce one of its major adverse side effect of Diacerein induced diarrhoea or soft stool and yellow color urine[2-7].

MATERIALS & METHODS

For the present study Diacerein Mfgd by: M/s Elder Pharmaceuticals Ltd., A-36, MIDC, Industrial area, Patalganga, Mumbai was obtained as free gift. HPMC, Ethyl cellulose, MCC were from S. D. fine Chemicals Ltd. All other chemicals were of A. R. grade manufactured by Merk Ltd. and Sigma Aldrich.

Instruments used:-

* Mini Lab Coater, Manufacturer- Umnag Pharmatech Pvt. Ltd
* HPLC – Perkin Elmer (Model Flexar)
* Dissolution apparatus, Make Lab India, model DS 8000+
* IR – Shimadzu (Model Prestige 21)
* Differential scanning Calorimetry (Model DSC 4000, software Pyris)

Manufacturing process of Diacerein Microsphere:-

1st Stage: Preparation of Drug pellet:

Quantity of Dummy pallets taken for coating: - 300.0 gm.(size #18-20)

Annxure-3

microenvironment. This typical environment has a correlation with cancer aggressiveness including increased angiogenesis, invasiveness, metastasis and chemo-resistance.

Cancer cells adapt and survive in the hostile acidic environment by the over-expression of proton pumps that extrude protons out of the intracellular environment. It is evident from literatures that cancers with low metastatic potential utilize mostly Na+/H+ exchangers, whereas V-ATPases are used for highly metastatic cancers. Therefore, the manipulation of acidity of the tumor microenvironment by the use of PPIs (V-ATPase inhibitor) represents a novel therapeutic strategy for the prevention of cancer. PPIs can exert direct cytotoxic effect with higher rate of apoptosis by increasing cellular caspase activity and early accumulation of reactive oxygen species within the cell. It is associated with the inhibition of mTOR signaling which is a major regulator of cell growth and autophagy. Besides implementing direct toxic effects, PPIs can reverse multi drug resistance of major anticancer drugs by increasing the pH of the microenvironment. It has shown that taking the advantage of tumor acidic microenvironment, PPIs with PTX can be utilized as a potent therapeutic combination. As an effective drug delivery system, the hypothesis of introducing the beneficial effect of PTX in combination with LAN as a single nanocarrier system with a biodegradable PLGA carrier matrix can enhance therapeutic efficacy, reduce toxicity in a hostile condition.

INT-CONF BA/BE P193

Development evaluation and pharmacokinetic studies of Diacerein microsphere

SoumenMukhopadhyay[1] and Tapan Kumar Chatterjee[2]

1. Division of Pharmacology, Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032.
2. Prof of Pharmacology (retired), Department of pharmaceutical Technology, Jadavpur University, Kolkata -700032, Dean Department of Pharmaceutical technology, JIS University, Kolkata-700109. Email: msoumen912@gmail.com

Diacerein is a drug under the class of anthraquinone derivative, it is an anti inflammatory drug used in the treatment of arthritis and osteoarthritis. Chemically Diacerein or diacetylrehin is 4,5-Diacetoxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid. It is a relatively short acting drug, practically insoluble in water having bioavailability of 35-56%.

Diacerein loaded microsphere was prepared using spray coating (wurstermethod) with a view to manufacture sustained release formulation. Assay by HPLC, Drug loading and entrapment efficiency were calculated. Particle size, Size distribution, Zeta potential, Poly disparity index were measured by Dynamic Light scattering technique. Shape and surface morphology were determined by Scanning Electron Microscopy (SEM study). FTIR study, X-Ray diffraction and Differential Scanning Calorimetry (DSC) study were done to establish drug polymer and other exihipients compatibility and stability. In vitro dissolution rate study of Diacerein microsphere was carried out in phosphate buffer (pH 6.8) and Citrate buffer (pH 6.0) to establish its sustained release action and compared with drug release from Diacerein capsule available in the market.

Pharmacokinetic parameters like Cmax, Tmax, AUC 0- t, AUC o-^, $T_{1/2}$, Kel of Diacerein microsphere in rabbit plasma were determined and compared with marketed capsule formulation. Good in-vitro and in-vivo correlation was seen.

CHAPTER-13

PHARMACOLOGICAL ACTION

As per recommendation of external examiner of PhD thesis, revised version is submitted herewith.

This revised version contains pharmacological evaluation of diacerein Microsphere in animal model like rat and mice.

Name of the Scholar : Soumen Mukhopahyay

Name of the Supervisor : Prof. Dr. Tapan Kumar Chatterjee

Former Professor, Dept, of Pharm Tech, Jadavpur University, Kolkata-700032.

Registration No of Research Scholar : D-7/E/516/15 dated 21.08.2015.

Index No. : 234/15/Ph.

Dt of Submission of PhD thesis : 05 July, 2021.

Evaluation of Pharmacological action Diacerein Microsphere formulation for its anti inflammatory and anti arthritic action in animal model.

1. Peripheral analgesic activity like writhing test on mice.
2. Croton oil induced acute inflammation in mice.
3. Cotton pellet induced granuloma in Rats.
4. Formalin induced Paw edema.
5. Evaluation of anti arthritic activity of Diacerein Microsphere in Rat model.
6. Carrageenan – Induced Paw edema.

1) Peripheral analgesic activity like writhing test on mice (i) (ii).

Writhing test is a chemical method used to induce pain of peripheral origin by injection of irritant principals like phenylquinone, acetic acid in mice.

Here the test compound is Diacerein microsphere (147.1 mg/gm). Analgesic activity of Diacerein Microsphere is inferred from decrease in the frequency of writhing.

Total eighteen mice were taken for test and divided into three groups each containing six mice in one group.

Gr. 1 six mice were injected normal saline through intra peritoneal route served as control group.

Gr. 2 six mice were injected acetic acid (0.5%) through I.P. route as test 1 group.

Gr. 3 six mice were injected Diacerein Microsphere solution in DMSO and phosphate butter (1:1 ratio) PH 7.2 solution through same route. Drug concentration was maintained as 50 mg/kg as test 2 group.

Writhing generated by I.P. administration of acetic acid in Gr. 2 caused profound pain of endogenous nature which recur for a prolonged period of time. Due to irritant in nature, these principles are prone to induce lesions. Writhings is an overt response to intense pain induced by irritant principles via nociceptors characterized by episodes of retraction of abdomen and stretching of hind limbs. The signals transmitted through CNS in response to pain due to irritation caused release of mediators such a prostaglandin.

In Gr. 3 where test compound Diacerein Microsphere solution was used caused less contraction of abdomen muscles in mice, less stretching in hind limbs as compared to Gr. 2 animals. Which shows analgesic activity of Diacerein Microsphere.

Whereas normal saline treated mice of Gr.1 Showed no abnormal response like irritant pain.

Inference : This study shows analgesic activity of Dicerein Microsphere as of NSAIDs. Diacerein in the form of microsphere as drug delivery is the first line treatment in osteoarthritis and the drug is devoid of Gr.1 toxicity, renal and cardio toxicity compared to NSAIDs. Though writhing test was highly criticized by the animal activist group because of its cruel nature. But the test is an easy way of understanding the analgesic activity of a drug in qualitative manner.

2) Croton Oil-induced acute inflammation in mice (iii), (iv)

Ear edema caused by croton oil or its irritant Printcipal 12-0- tetradecanoylphrbol – 13-acetate (TPA) has been extensively used to access the anti inflammatory activity of steroidal and non steroidal anti inflammatory drugs. The protocol is designed to detect the inflammatory/anti inflammatory potential effects of histamine ligands in a model of acutre skin inflammation induced by local application of croton oil. The histamine H_1 receptor antagonist pyrilamine 30 mg/kg. and dexamethasone (2 mg/kg) represent the reference anti inflammatory drugs administered subcutaneously.

a) Material and reagents

- Male CD-1 IGS mice, aged 6-8 week (20-25g body weight)
  (They are outbread mice derived from a group of outbred
  Swiss mice developed at the anticancer centre of
  Switzerland).
- Animal balance accurate to 1gm (Sartorius)
- Analytical balance accurate to 0.1 mg.
- Surgical instruments: Sharp and blunt scissors, small straight anatomical Forceps.
- 50-100 m! glass breakers.
- 200-1000-2000 µl Pipettes (Micropipette)
- Magnetic Stirrers.
- Vortex mixtures.
- Croton oil (2.5% in acetone)
- Diacerein microsphere in normal Saline Suspension.
- Compounds are to be prepared immediately before use.

b) Animal preparation

- 50 CD-1 mice are to be kept in Standard Condition at 23-25° C temperature, RH = 55±10% and 12 hr light and dark cycle in the experiment room.
- Each mouse was weighed accurately in animal balance accurate up to 1 gm.
- Mice were weighed randomly in single doses of test compound or vehicle.

c) Croton oil induced ear inflammation:

- Animals were kept in fasting Condition for 18 hr before the start of experiment with free access of water. Experiment conducted between 10 am to 3 pm in order to avoid the influence of circadian Variations in Corticosteroid levels in inflammatory responses.
- Test animal mice was kept 6 per group. Total 18 mice were taken in three groups. One group was control group, other two groups were test group for induction of test formulation Diacerein microsphere and Diacerein Ph.Eur (DCN).
- DCN has a solubility of approximately 0.5 mg/ml in a 1:1 solution of DMSO:PBS (P H 7.2) [0.010 mg/,l] (PBS-Phosphate buffer Solution PH 7.4).
- Due to sparingly soluble in water DCN was first dissolved in DMSO (dimethyl sulfoxide) and further diluted with Phosphate buffer to make a 1:1 mixture (PH7.2).

In the same way 0.5mg/ml solution of DCN microsphere, DCN pure drug was prepared. Since DMSO reduces croton oil induced edema (approximately 40%). Test compound DCN microsphere and DCN pure drug was first dissolved in a vehicle Containing 20% DMSO and 80% 2-hydroxypropyl-β-cyclodextrin in amounts which did no change in ear swelling in itself.

- Both Control and test group of mice containing six in each group were injected normal saline in control group and two test groups were injected DCN pure drug solution and DCN microsphere solution 0.5mg./ml through SC route and intra peritoneal rough (3ml each).
- Cutaneous inflammation was induced in two test groups of conscious mice by topical application of Croton oil (2.5% in acetone) with a micropipette to the (20µl/ear) inner surface of right ear. Acetone was applied to the left ear which serves as marker of uninflamed ear.
- After 4 hours of croton oil application, all mouse were killed by cervical dislocation. Immediately thereafter both croton oil induced ear and only acetone induced ears of mouse were cut horizontally, across the indentation at the base of the ear.
- Difference in the Wright of inflamed ear and uninflamed ear were measured in mg. unit.
- Average weight of inflamed ear of croton oil induced group of six mice was compared.

- DCN microsphere solution induction versus DCN pure drug. Solution induction and control group ear weight were compared.
- It was observed that Croton oil application in six mice where DCN microsphere solution was injected and where DCN pure drug solution was injected had only 10-15% variation in weight (mg) Similarly uninflamed ear of the above two groups also had 20% variation in weight in DCN microsphere solution injected group and DC N pure drug solution injected group.
- Control group of six mice where normal Saline was injected showed sum of weight of ear nearly equal to acetone induced group of mice.

Inference:

Topical application of Croton oil produces vasodialatation and increases vascular permeability and it also causes an influx of neutrophil. It may cause eicosanoids synthesis and histamine and Serotonin may be released. The Croton oil ear test is widely used to indentify prospective topical anti inflammatory drugs also. Generally rats and mice are the experimental animal models used for this test.

3) Cotton Pellet induced granulome in Rats

Diacerein, known inhibitors of cytokines like IL-Beta, IL-6,TNF-α Tumor necrosis factors, inteferons's interleukin and colony stimulatory factors belong to the class of cytokines.

Pharmacological action, therapeutic effectiveness, toxicity of Diacerein microsphere can be measured by different animal models.

Cotton Pellet-induced Granuloma is a well established method for testing of anti inflammatory activity. This model represents the pathological events in chronic inflammation which is represented by monocyte infiltration, fibroblast proliferation, angiogenesis and exudation. The transudative and proliferative elements of the chronic inflammation are evaluated through this model.

Anti inflammatory activity of Diacerein microsphere in carried out by granuloma tissue formation method through Cotton pellet grafting under skin.

Male albino rats (150-225g) were experimental animal used. Rats were acclimatized to laboratory condition well before the Commencement of the experiment and kept at 23 to 25°$_C$ temperature in air conditioned room. Relative humidity of the room was maintained at 75±10% in the room and half light and half day dark cycle was maintained in the room. Institutional Ethics Committee permission was obtained prior to the Conduction of the study, involving animal sacrifice.

Experimental rats were kept in fasting Condition 24 hrs prior to experiment, however water was given ad libidum. Rats were divided in to five groups.

With six rats in each group, one group of six rats was kept as Control with 1% CMC solution. Two groups were kept as standard groups and they were given Diacerein Ph Eur suspension in normal Saline using 50, 75 mg/kg. body weight via oral route.

Two groups were kept as test group and given Diacerein microsphere suspension and normal saline in Same Concentration as Standard group via oral route. The animals were anaesthetized with diethyl ether. Hair of the back skin of lumber region was removed and disinfected with rectified spirit. Using stainless steel Scalpel a subcutaneous lecision was made by the Scalpel. Sterilized cotton Pellet with 1% Carrageenan Solution in normal saline was implanted in the lesion, the cotton pellet was weighing between 60-80 mg. The lesion of the back skin of the rats were closed with Stainless steel suture clip, separate cages were allocated for animals in group wise. Through oral gavages Diacerein Ph Eur and Diacerein microsphere suspension were introduced to the peroral route for six days. On the seventh day animals were scarified under anesthesia and cotton pellets surrounded with granuloma tissue were removed. Weights of the wet cotton pellets were taken and dried. Then they were dried at 60-65°$_C$ temperature until constant weight of the dried pellets were taken and recorded. Net weight of the dried pellets were taken and recorded. Net weight of the Cotton pellets were taken and recorded. Net weight of the Cotton pellets were calculated by subtracting weight of the dry cotton pellets from the weight of the wet Cotton pellets.

Table-I

Comparative Granuloma tissue weight of implanted Cotton pellets in rats.

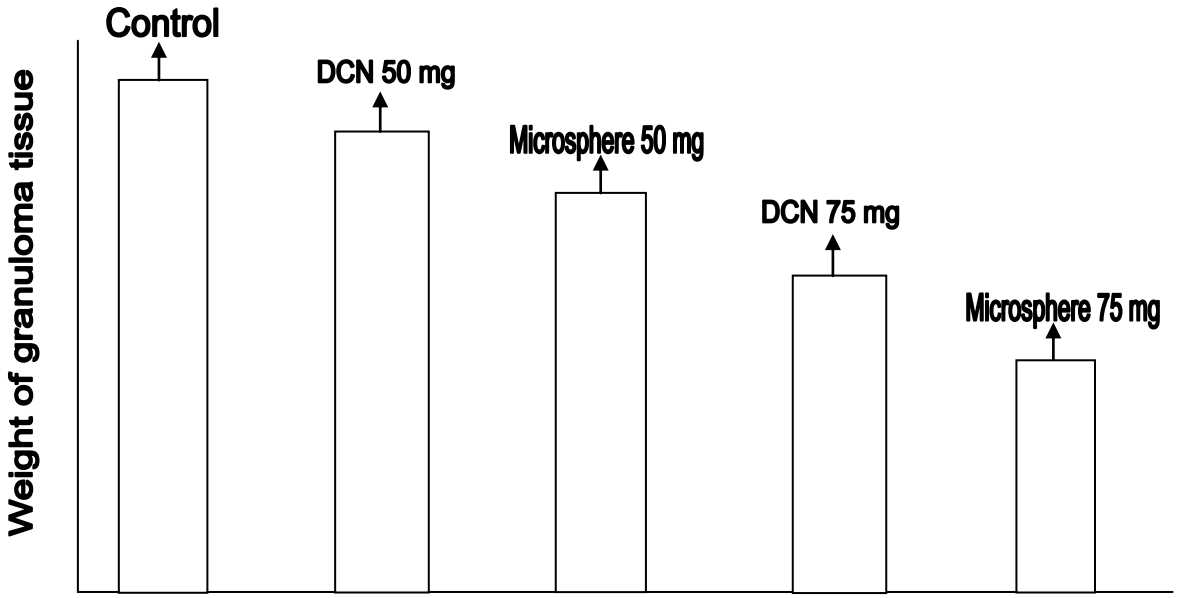
Treatment tissue weight	Dose (Peroral, mg/kg body weight)	Granulma In mfg ± SD
Control	-	128.21± 5.012
Discerein Ph Eur	50 mg	110.11±5.012
Diacerein Microsphere	50 mg*	98.7 ±5.015
Diacerein Ph Eur	75 mg	75.21±6.852
Diacerein Microsphere	75 mg*	65.52±6.251

- Dose equal quantity of Diacerein present in microsphere, $P<0.01$ compared with control group, Table value (df 4, 25)=2.76 at 0.05 level and 4.18 at 0.01 level respectively. The results were analyzed by one way ANOVA followed by Dennett's Multiple Comparison test.
- Each value represents the mean ± SEM of six rats. Granuloma tisse formation is one of the main characteristics in chromic. "Inflammatory Conditions".

For screening of anti inflammatory drugs, animal models of granuloma tissue formation can be employed for the screening of anti inflammatory drugs. The cotton pellets test was first described by Meien, Schuler, Desulls (1950) for the evaluation NSAIDS. In case of Discerein Ph Eur treted rats (50,75 mg/kg b.w) , this average of weights of Cotton pellets were higher in comparison to diacerein microsphere treated rats of 50 or 75 mg/kg b.w respectively (Table-I). This may be probably that Discretion microsphere delivered the drug for a longer period of time (around 16 hrs) at the inflammatory sites of treated rats. As a result the granuloma tissue formation was reduced in Diacerein microsphere trerated groups. The graphical representation of cotton pellet test was Shown in Fig. I. The results of Cotton pellet test suggested that Diacerein microsphere sustained release formulation was more efficacious in treatment of chronic pain in inflammatory Conditions due to its longer duration of action.

Fig – I

Cotton pallet test Control



Graphical representation of comparative Anti inflammatory efficacy by Cotton pellet implantation method in rat.

4) Formalin – Induced paw Edema in animal model (ix)

This model has close resemblance with human arthritis. It is considered as the suitable experimental model for the assessment of chronic anti-inflammatory effects of various agents. Formalin induced inflammation is biphasic. The early neurogenic phase is conciliated by bradykinin and substance P whereas the later inflammatory phase shows the involvement of histamine 5 HT, prostaglandins and bradikin. The CNS acting drugs like opioids suppress both the phases. While the drugs acting through peripheral nervous system like NSAIDs and corticosteroids exclusively inhibit second phase.

Inflammation :

It is a reaction of the living tissue towards injury comprising of systemic and local response. It can also be termed as complex biological response of vascular tissue to harmful stimuli such as irritants damaged cells, pathogens etc.

Inflammation is a protective response to remove injurious stimuli and also a healing process. On the other hand chronic inflammation is by Granuloma formation study of inflammation is by granuloma formation study of inflammation is by different animal models.

These in-vivo models are

i) Paw edema in rats.

ii) Croton oil ear edema in Rat and mice.

iii) Granuloma pouch technique.

iv) Oxatolone – induced ear edema in mice.

Measurement of proliferative phase is done by testing granuloma formation.

Cotton wool granuloma, Glass rod granuloma, PVC sponge granuloma.

5) Title: Evaluion of anti arthritic activity of Diacerein Microsphere in Rat Model (viii)

Diacerein is an anti inflammatory and drug used in the treatment of arthritis and Osteoarthritis as defined by BP(2022), IP(2022) also defined Diacerain as antirhumatic. The present study aimed to determine beneficial effect of Diacerein in Cartilage protection in chemically induced Osteoarthritis in rat as animal model.

Freund's complete adjuvant (FCA) can induce arthritis in rats.

OA is most Common among all arthritis its prevalence in the elderly population and it may cause disability. Therapy of OA is mostly palliative and no pharmacologic agent has been shown to prevent or delay the progression of OA or reverse the pathologic change in OA in human being. Though NSAIDS are the 1st line of drug in OA but it's long term use can cause cardio, nephro and hepatotoxicity. Many clinical studies on Diaceren have shown it effectiveness to reduce the Symtoms of OA. Studies on mouse granuloma model have shown that Diacerein can prevent cartilage break down. In canine model of OA, Diacerein slows down the progression of cartilage lesions.

Diacerein is completely metabolized to rhein in human body and also in animal. There are various methods by which diacerein and its active metabolite rhein acts but the exact mechanism for anti inflammatory effects is not clearly known. Chronic inflammatory model that is anti arthritic effects of Diacerein in FCA induced arthritis in rats is studied here –

Material and Methods :

i) Weighing Scale

ii) Plethysmometer

iii) Animal Cages

iv) Mouth Gag

v) Syringe

vi) High Power Microscope

vii) X-Ray Apparatus.

The drugs used in the experiment were FCA purchased from Sigma Aldrich (each ml of FCA contain 1 mg mycobacterium tuberculosis (H37 Ra, ATCC 25177) heat killed and dried, 0.85 ml paraffin oil, 0.15 ml mannide mono oleate, Diacerein microsphere (147 mg/gm), Diclofenac Sodium (voveran). Both the drugs were dissolved in normal saline and normal Saline was used as control, ketamine.

To evaluate whether diacerein prevents arthritis and suppresses joint destruction in vivo, this prophylactic treatment model was followed where arthritis was induced on day "O" by FCA and drug treatment was also started simultaneously from day O to day 21/ Diclofenac Sodium was used as standard anti inflammatory drug for comparison and normal saline was taken as control.

The animals were numbered, divided into five groups with six animal in each group and kept in separate cages and and cages were numbered.

The basal body weight and hind paw volume of both right and left paw of all animals noted on day '0' and body weight and hind paw volume of right and left Paw of all animals noted on days 4^{th}, 8^{th}, 14^{th} and 21^{st}. Grouping of animals were done. Different doses of diacerein (50mg/kg 100 mg/kg, 200 mg/kg), diclofenac Sodium (5mg/kg)or vehicle (normal saline 1 mg/ml) were administered orally once daily from day '0' to day '21'. Normal control group (Group (1): Animals in this from group were treated with normal Saline solution peroral.

Diclofenac Sodium treated gr.(ii) where dose of 5 mg/Kg was used.

Diacerein microsphere treated gr.(iii) 50 mg/kg dose was used and in the same manner in gr. (iv) 100 mg/kg and in gr (v) 200 mg/kg dose of Diacerein microsphere was used.

Assessment parameter :

To study the course of the disease and to observe the effect of drugs on adjuvant induced arthritis in, rats the swelling of adjuvant injected hind paw was determined on day 'o' and the development of arthritis as indicated by increasing Paw volume measured by volume development by Plethysmograph (It is used to measure changes in volume in different parts of the body) and also the body weight of the animals was measured on 4^{th}, 8^{th} and 21^{st} day to confirm the reduction in arthritis.

a) Paw volume evaluation in ml :

Edema was recorded with respect of basal paw Volume and percentage inhibition of Paw edema with respect to the Control group was calculated as Mean change in Paw volume from the basal on the respective days and percentage inhibition of Paw volume from Control.

% inhibition of edema with respect to untreated group is gien by the formula.

$$\mathbf{i = 1 - \frac{\Delta V \text{ treated}}{\Delta v \text{ untreated}} \ X\ 100}$$

where i is the % inhibition of Paw edema.

ΔV Treated = mean change in Paw volume in treated rat.

ΔV Untreated = mean change in Paw volume of untreated rat.

b) Body weight : Basal body weight of each animal was measured the help of pre calibrated weighing balance, before injecting CFA. Mean body weight in grams and Percentage change in body weight from basal Calculated for the respective days (4^{th}, 8^{th}, 14^{th}, 21^{st}).

2. Radiographic assessment of joint damage : Radiographic assessment and evaluation of joint damage.

After completion of the study period on day 22, the animals were anesthetized with ketamine and placed on the X-ray plate. X-ray of both right and left ankle joint of each rat from Control, Diacerein microsphere and Diclofenac Sodium treated groups done and compared. X-ray apparatus Amedeo P- 100/20 HB - battery operated portable X-ray machine

(monoblock), power requirement 5.0 KW with 0.1-100 mAS with a 54 V peak, 0.25 exposure time was used for radiographs.

3. Histopathological analysis :

22nd day rats were anesthetized and scarified by application of ether. Paws of rats were dissected out for histopathological examination. Sections were fixed in 1% formation, decalcified, sectioned and stained with hematoxylin and eosin to study the histopathological Changes in all the groups under light and high-powered microscope.

Statistical Analysis:

The data were analyzed using statistical package for the social sciences (SPSS) Version 20. Paired and unpaired t-test was done. A p-value of less than 0.05 was Considered to be statistically significant.

Results:-

Maximum inhibition of paw volume with Diclofenac Sodium 5mg/kg in the Freund's Complete adjuvant induced arthritic paw volume was from 8th to 14th day and with Diacerein microsphere inhibition of Freund's adjuvant induced arthritic Paw volume was on the 21st day with 100 mg / kg [Table/fig 1]

There was a decrease in body weight due to Freund's adjuvant in normal Saline treated group from 4th to 21st day of observation. In the Diclofence Sodium treated group there was also greater decline in body weight. from 4th to 21st day in contrast in the Diacerein microsphere treated group there was an increase in body weight from 4th to 21st day in all the three doses [Table/ Fig 2]

On day 22 normal rat paw showed no soft tissue swelling with normal joint space and no increased opacity or sclerosis [Table/ Fig 3], but adjuvant induced arthritic Paw showed soft tissue swelling, Sclerosis of bone around joint sclerosis increased opacity and the joint space is barely visible with reduction of joint space [Table/ fig 4].

Similarly, on 22nd day, Diclofenac Sodium 5mg/kg treated rat paw showed soft tissue. Swelling, grossly decreased joint space, increased opacity of joints showing bony sclerosis [Table / fig5] but on day 22, treatment with Diacerein microsphere 100mg/kg it was observed that Soft tissue swelling was markedly less, joint space is normal and the picture of normal rat paw [Table/fig7]. On histopathologic examination with Haemotoxylin and eosin Staining

and 40 × magnification under microscope, normal bone marrow with adipocytes observed. Bony spicules visible with no inflamatory infiltrate and bone layer is intact [Table/ Fig. 8]. Picture of adjuvant induced arthritic rat paw on day 22 [Table / Fig 9) and histopathologically of the same joint shows disruption of bony spicule margin, Sourrounding the bony spicule there is a Zone & inflammatory cells and The bony layer is thickened of places and may denote bony sclerosis [Table/fig 10]. The picture of Diacerein microsphere 100mg / kg treated rat paw on day 22 [Table/fig 11] and on taking histopathological section of same it was seen the cartilaginous lining intact, intact bony spicule was visible with no sourrounding zone of inflammation, very little inflammatory infiltrate and Bone marrow is normal (Table/fig 12]. Picture of Diclofenac Sodium 5mg/kg treated rat paw [Table/fig 13], the histopathologic section of which Shows surface of bony layer is some what disrupted and Sourrounding the bony spicule in a layer of inflammatory infiltrate with a lot of inflammatory cells[Table fig 14]

Discussion

Osteoarthritis is not merely a disease of cartilage but all the tissues of the joint, including the Synovium, Subchondral bone, particular muscle and supporting ligaments are affected in the disease Cartilage damage is the crucial feature of the disease irreversible in nature . Accordingly the drug that block the destruction of cartilage will be of therapeutic value. currently the primary approach in the the treatment of OA involves use of NSAIDS- Because of inadequacy of present day drug therapy underline the need to improve technical feasibility of discovering Safe and and effective antiarthritic agent Diacerein is an effective treatment of OA. The present study was undertaken on animal model to further obtain insight into the anti arthritic properties of Discerein in the form of microsphere formulation as drug delivery. Diacerein microsphere in doses 50,100, 200 mg/kg is I-L-1 Beta inhibitor. Rhein is an anthrquinone found in plants of the genus Cassia and has moderate and inflammatory and analgesic property, weak laxative effect.

The form of arthrities produces by injection of microbial particles into rats is called adjuvant induced arthritis. It was induced by killed mycobacterium not limited to joints, but has extra articular manifestations including tendonitis, iritic, modular lesions in the visceral organs, arthritis and diarrhoea. The Symptoms become peak after 10 to 15 days after injection. followed by slow resolution. The synovial lesions when viewed under microscope are similar to those observed human rheumatoid arthritis. The rat adjuvant induced arthritic model is

widely used for the evaluation of NSAIDS drugs and antirheumatic drugs, Adjuvant induced arthritis is the only experimental model which has been considered to as parallel to human arthritis disease more closely than any other laboratory model. Diacerein microsphere when administered from 0-21 days in doses 50mg of Diacerein per kg /100 mg/kg produces significant reduction in arthritic paw volume starting from 4th day to 21st day of observation while Diacerein microsphere equivalent to 200mg of Diacerein per kg produced this effect from 8th to 21st day of observation (Table/ fig 1) Diclofenec Sodium 5 mg/kg significantly reduced the paw volume from 4th day of observation to 21st day of observation [Table / Fig 2]. Maximum percentage inhibition of paw volume was seen with Diacerein mocrosphere 100mg/kg at 21st day of observation while with Diclofenac Sodium it was seen on 14th day of observation.

Similar fininding was reported by (Tamura T et al) when Diacerein was administered orally in Rats for three weeks with adjuvant induced arthritis it significantly inhibited the swelling of the injected and non injected paws in Rats at the dose of 100mg/kg . [Tamura T, Shrai T and others Pharmacologic studies of Diacerein in animal model of inflammation, arthritis and bone Sorption, Eur. J. Pharmacol - 2002); 448 (1): 81-87]

Study showed here that all the three doses of the Diacerein microsphere increased the body weight of Rats significantly in comparison to basal from. 14th to 21st day which Diclofenac Sodium could able to do.

Both the Control and Diclofenac Sodium5mg/kg treated group a significant decrease in body weight was observed from 14 to 21 days [Table/fig 2]. Change in body weights in Rats have been used to assess the cource of disease and the response of therapy with anti inflammatory drug. During the course of therapy change in body weight of Rats occurred at the experimental period.

The increase in body weight in Rats during treatment with Diecerein microsphere may be due to restoration of absorption capacity of Intestine.

Conclusion

FCA is an effective anti arthritic model which increases progressive increase in paw Volume and reduction of body weight as a part of systemic involvement.

Diacerein microsphore in all the three deses 50/100/200 mg/kg when given orally for three Weeks as prophylaxis showed significant anti arthritic action as evident from inhibition of the

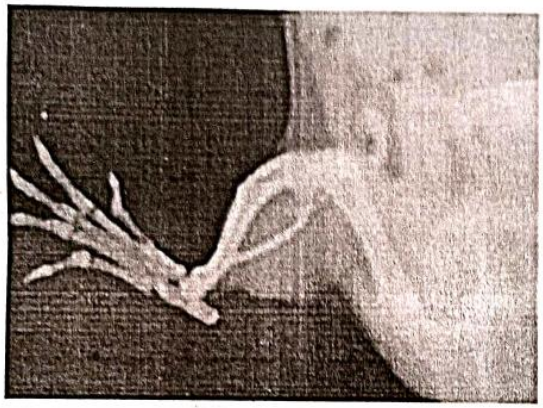
swelling of paw volume in FCA injected experimental Rats and also Causes increase in body weight in comparison to basal Values, So Diacerein microsphere formulation possesses antiarthritic and anabolic effect in animal model can be a significant agent with disease modifying effect in the treatment of OA.

Groups	Drugs dose (mg/kg)	Mean change of right hind paw volume from basal (in ml) ±SEM (%inhibition of paw volume from control)				
		Baseline	4th day	8th day	14th day	21st day
(Control)	Normal Saline (1 ml/kg)	1.11±0.03	1.86±0.02	1.11±0.03	0.55±0.04	0.2±0.06
II (Standard)	Diclofenac Sodium 5 mg/kg	1±0.01	0.65±0.10*** (65.16)	0.04±0.03***(96.4)	0.016±0.01***(97.09)	0.03±0.04***(83.[illegible]
III	Diacerein microsphere 50 mg/kg	1.06±0.02	0.31±0.03***(83.04)	0.13±0.03 (88.09)	-0.01±0.01***(102)	-0.06±0.03**(130)
IV	Diacerein microsphere 100 mg/kg	1.05±0.02	0.21±0.04***(88.5)	0.01±0.04***(100)	-0.02±0.09* (154.36)	-0.56±0.15** (380)
V	Diacerein microsphere 200 mg/kg	1.03±0.02	0.2±0.03 (84.9)	0.05±0.03***(95.52)	-0.05±0.03***(109)	-0.4±0.012**(300)

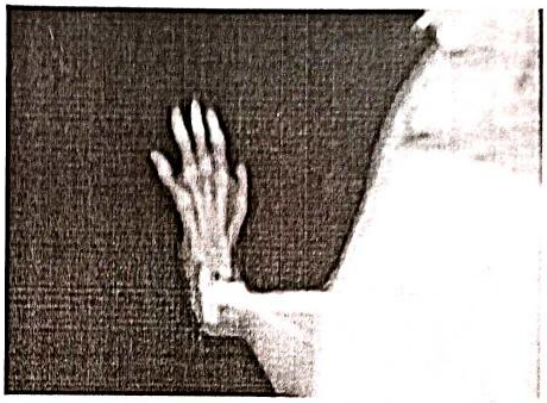
[Table/Fig- 1]: *Change in paw volume in adjuvant induced arthritis in rats in prophylactic model by drugs. n=6. Statistical analysis done from control values by Unpaired t-test, *p<0.05, **p<0.01, ***p<0.001*

Groups	Drugs dose (mg/kg)	Mean body weight (in gm) ±SEM (% change in body weight from baseline)				
		Baseline	4th day	8th day	14th day	21st day
I (Control)	Normal Saline (1mL/kg)	129.5±2.47	128±2.4 (-0.54)	127.5±2.2** (-1.54)	125.3±2.2***(-3.24)	123.8±2.2***(-4.4)
II (Standard)	Diclofenac sodium 5 mg/kg	114±6.8	112.1±6.9***(-1.75)	109.08±6.5*** (-4.31)	100±6.1***(-11.99)	98.66±6.5**(-13.45)
III	Diacerein microsphere 50 mg/kg	90.33±4.9	91.16±4.9*(0.09)	85.38±11.2** (5.4)	95.5±5.17***(5.72)	96.83±5.05***(7.19)
IV	Diacerein 100 mg/kg	78.9±4.97	79.08±4.71(0.22)	77±2.5(2.4)	80.1±3.07***(1.52)	81.71±3.3 (3.56)
V	Diacerein microsphere 200 mg/kg	94.1±3.8	94.83±3.9* (0.77)	96.16±3.80 (2.18)	97.33±3.71***(3.43)	98.16±3.55***(4.31)

[Table/Fig-2]: *Effect of drugs on body weight in adjuvant induced arthritis in rats in prophylactic model. n=6. Statistical analysis done from Basal Values by paired t-test, *p<0.05, **p<0.01, **p<0.001*

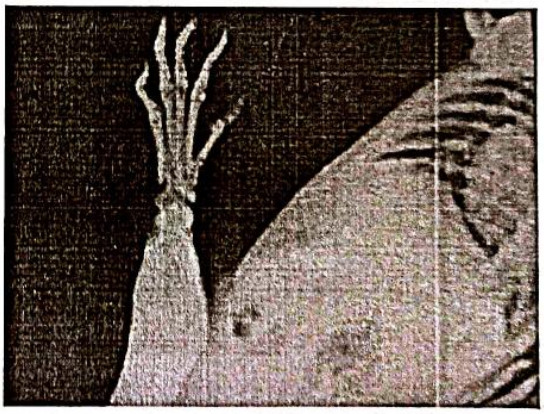


[Table/Fig-3]: Radiograph of normal rat paw on day 22.

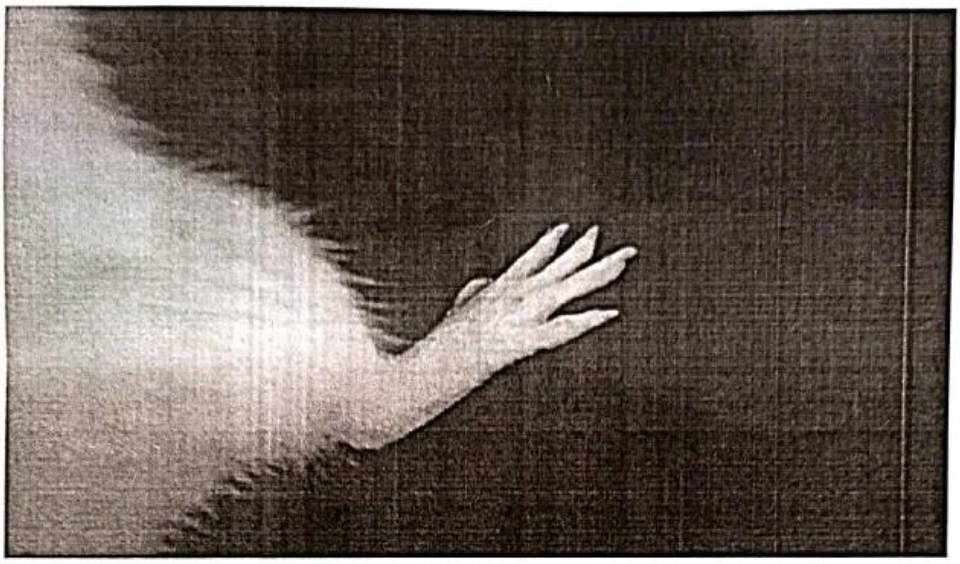


[Table/Fig-4]: Radiograph of adjuvant induced arthritic paw on 22nd day.

[Table/Fig-5]: Radiograph of Diclofenac 5 mg/kg treated rat paw on 22nd day.

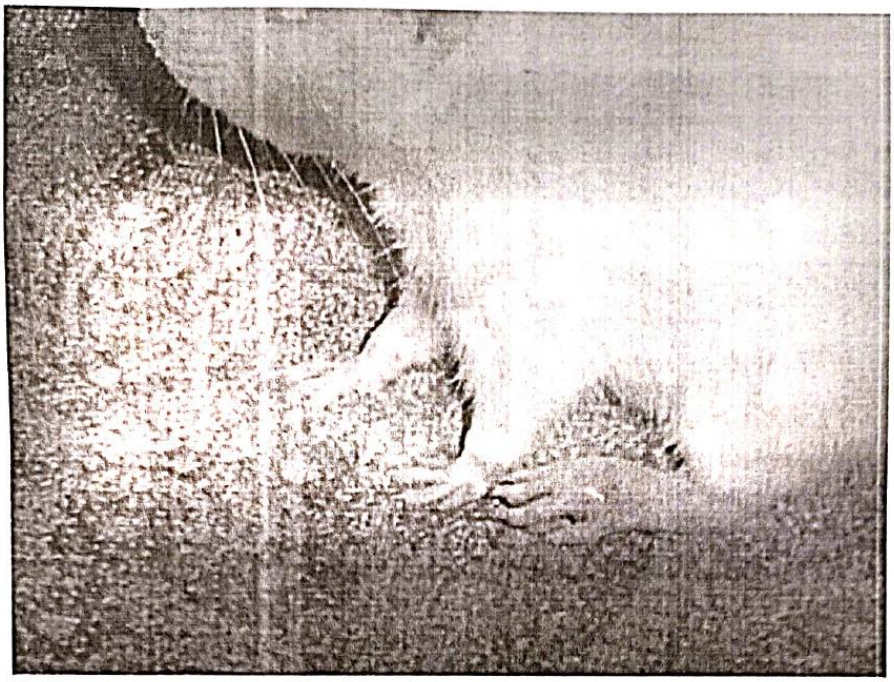


[Table/Fig-6]: Radiograph of Diacerein 100 mg/kg treated rat paw on 22nd day.



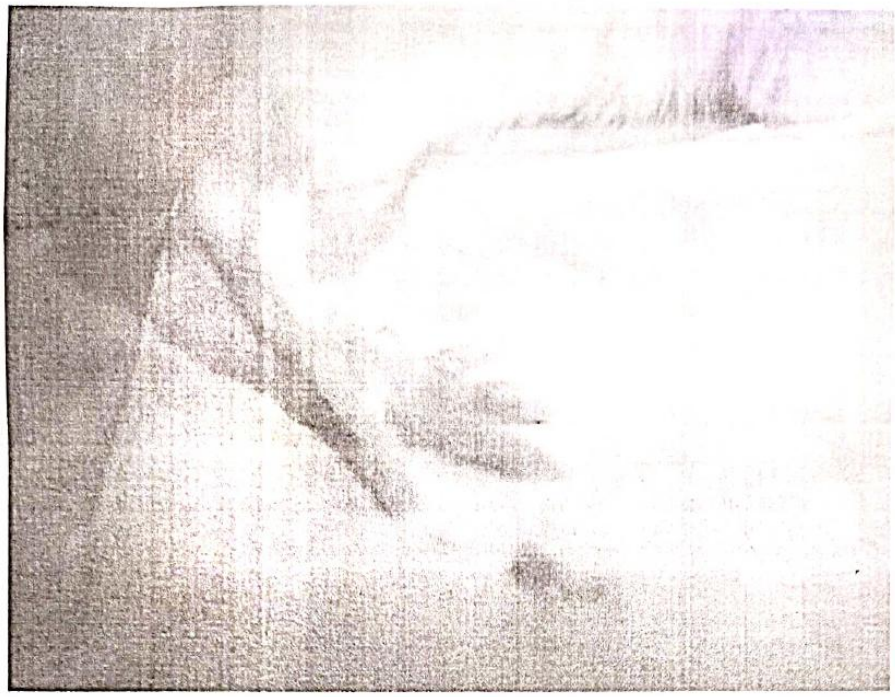
[Table/Fig-7]: Normal rat paw.

[Table/Fig-8]: Histopathological section of normal rat paw (X400).

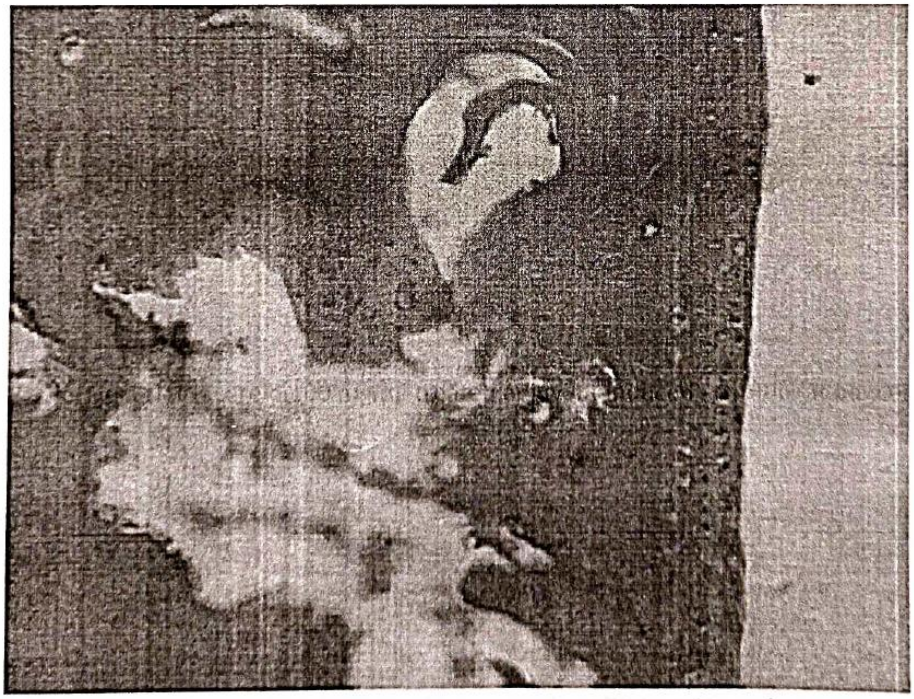


[Table/Fig-9]: Adjuvant induced arthritic rat paw on 22nd day.

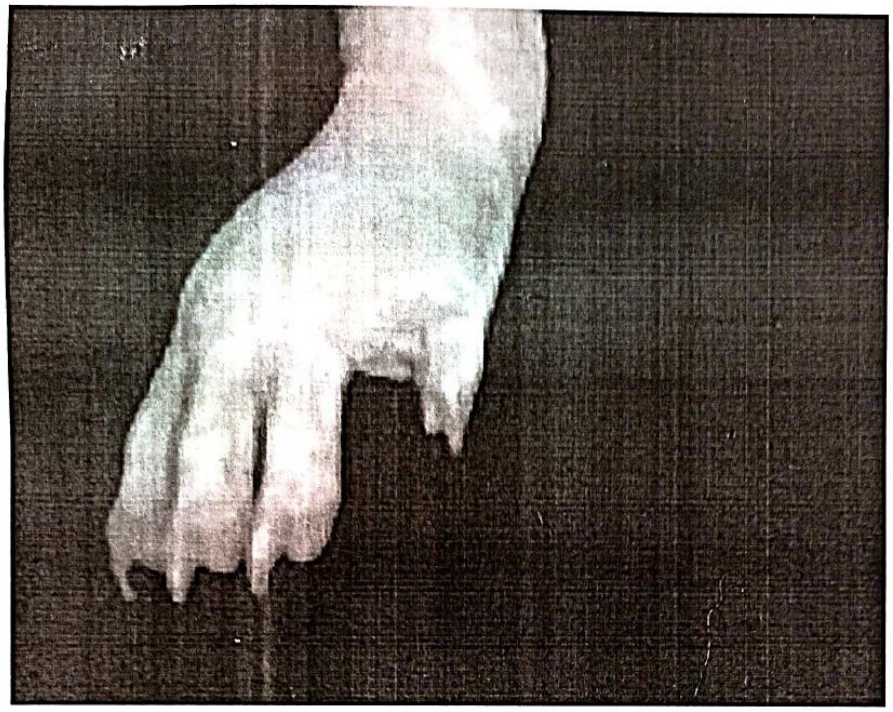
[Table/Fig-10]: Histopathologic section of adjuvant induced arthritic rat paw on 22nd day (X100).



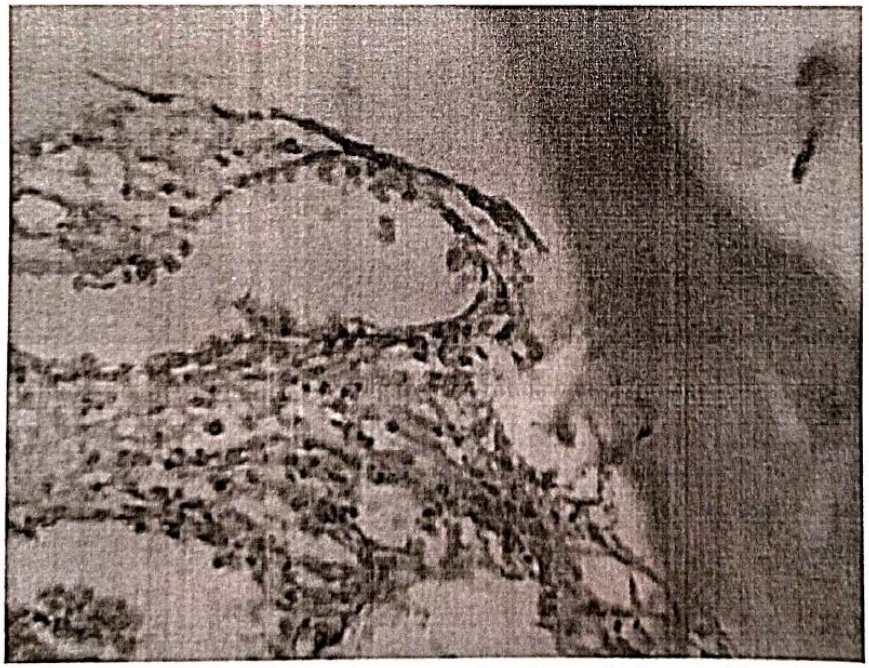
[Table/Fig-11]: Diacerein 100 mg/kg treated adjuvant induced arthritic rat paw on 22nd day.



[Table/Fig-12]: Histopathologic picture of Diacerein 100 mg/kg treated adjuvant induced arthritic rat paw on 22nd day (X100).



[Table/Fig-13]: Diclofenac 5 mg/kg treated rat paw on 22nd day.



[Table/Fig-14]: Histopathologic picture of Diclofenac 5 mg/kg treated rat paw on day 22nd day (X400).

6) Carrageenan-Induced paw edema inanimal model:

Carrageenan-Induced paw edema is a widely used and well established working model of the inflammation. Inflammation induced by carrageenan is acute, nonimmune and reproducible. Involvement of multiple mechanism allow this model as a preliminary test for screening of anti inflammatory drugs. Biphasic response after sub planter carrageenan injection enable this model to predict the prable biological targets of test drug in the inflammation. This model is sensitive to cyclooxygenase inhibitors and suitable for assessment of NSAIDS that act by cyclooxygenase inhibition which is involved in prostaglandin synthesis.

Limitation: To eradicate the effects of stress, animal should be acclimatized at least one week before commencement of an experiment. The experiment required proper training to the investigator to record the stable and reproducible paw volume using sophisticated equipment like Plethysmometer. Rise in paw edema is based on the concentration of injected carrageenan. Typically the maximum edema response produced by carrageenan is too difficult to inhibit. Therefore the carrageenan type and its solution needs careful attention.

Histamine/5-HT-induced Paw Edema :- Advantages: Histamine/ 5-HT- induced paw edema methods are convenient to appraise the acute anti inflammatory effect of substances. These models can be used secondary models to authorize the results of carrageenan induced paw edema model, especially for those drugs that showed effects at the first phase of carrageenan induced inflammation. These models are suitable for the assessment of those drugs that act through the histamine/or 5-HT inhibition.

Limitations: Inflammation or paw edema induced by Injection of histamine or 5-HT is minimal and transient. These models are rather inappropriate for the assessment of drugs like prostaglandin inhibitors, which act by the mechanisms excluding histamine and/or 5.

HT.

Bradikinin-Induced Paw edema

Advantages: It is an animal model of the acute inflammation. Results of this model can be correlated with the results of carrageenan induced paw edema model. Drugs inhibiting prostaglandins are effective in this model.

Limitations: Bradykinin produces only mild and transient edema.

Similarly there are Dextran induced paw edema, Lipopolysaccharide- Induced Paw edema, Arachidonic Acid-induced paw edema, Oxazolone-m Induced Ear edema models are useful as animal models of inflammation with advantages and disadvantages. There are scheme for the preclinical evaluation of the acute anti inflammatory activity.

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