Dissolution rate enhancement of Irbesartan using nanosuspension technology

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Abstract:

Irbesartan, an angiotensin receptor blocker, is a BCS class II drug, widely employed in the treatment of hypertension. The objective of this study was to formulate irbesartan nanosuspension to enhance the solubility and dissolution rate.

The nanosuspensions were prepared by solvent evaporation method and emulsion solvent diffusion method incorporating various ratios of stabilizers such as PVP K30, Poloxamer 188, HPMC E15, and Tween 80, using organic solvents like ethanol, dichloromethane and DMSO.

The best selected formulation, D2, was prepared by emulsion solvent diffusion method using PVP K30, Tween 80 and DMSO. Drug-excipient interactions in the nanosuspension were studied using Fourier transform infrared spectroscopy (FTIR). FTIR results showed that there were no drug-excipient interactions. The best selected formulation was further characterized for its particle size, zeta potential, Surface morphology and XRD. The average particle size was found to be 266.5nm and PDI was found to be 0.448. The zeta potential of -32.2mV indicates that the particles are negatively charged and the suspension was stable showing no signs of aggregation. XRD results showed that the crystalline state of drug got converted into amorphous form in the nanosuspensions. SEM results indicate that the particles in the nanosuspension were uniformly scattered, there was no aggregation, and the particles existed as individual entities and were discrete. The nanosuspension formulation released 94.29% of the drug in 10 minutes compared to pure irbesartan, which showed 25% release in 75mins.

Keywords: Nanosuspension, Irbesartan, Dissolution, Solubility.

Introduction:

Nanosuspensions represent a cutting-edge innovation in pharmaceutics, addressing the challenges of poorly water-soluble drugs. By engineering particles at the Nano scale, they dramatically enhance dissolution rates and bioavailability, not only due to the increased surface area but also through improved saturation solubility, facilitating efficient drug delivery even for compounds with challenging physicochemical properties. Furthermore, their versatility allows for diverse administration routes, including oral, injectable, and pulmonary, while enabling targeted delivery and controlled release, advancing personalized medicine. [4]

There are two primary approaches for preparing nanosuspensions: **Bottom-up technology** and **Top-down technology [5].** In **Bottom-up technology,** the drug is first dissolved in a solvent, which is then added to a non-solvent, causing the drug particles to precipitate [4, 5, 6]. This precipitation method is advantageous due to its simplicity, low cost, and the ability to achieve higher saturation solubility compared to other nanosuspension preparation techniques. However, it is not suitable for drugs that are poorly soluble in both aqueous and non-aqueous media, as the drug must be soluble in at least one solvent that is miscible with the non-solvent. A key challenge in this method is preventing crystal growth caused by Ostwald ripening, which occurs due to variations in saturation solubility between differently sized particles. [2]

The production of nanoparticles can result in either crystalline or amorphous forms, a mixture of both, or even a disordered phase. Amorphous drug nanosuspensions are particularly susceptible to particle growth due to Ostwald ripening.[2] To prevent this, a second component with extremely low aqueous solubility can be added to create a single-phase drug/inhibitor mixture, which inhibits the ripening process. Additionally, the high surface energy of nanosized particles tends to cause agglomeration of the drug crystals. This issue can be controlled by incorporating various stabilizing additives, which help ensure proper stabilization. The primary role of the stabilizer is to thoroughly wet the drug particles, preventing Ostwald ripening and agglomeration, and to create a physically stable formulation by forming a steric or ionic barrier [5]. Common stabilizers used in nanosuspensions include cellulosics, poloxamers, polysorbates, lecithin, polyoleates, and povidones. For nanosuspensions based on emulsion or microemulsion templates, selecting the appropriate organic solvent is crucial for developing an effective nanoparticulate formulation [3,4,5]. Key factors to consider when choosing an organic solvent include the solvent's physical properties and its ability to dissolve both the polymer and the drug [4]. Preferably, pharmaceutically acceptable, less hazardous, water-miscible solvents should be chosen over conventional, more hazardous solvents.

Irbesartan (Irb) nanosuspensions were prepared by high pressure homogenization method & media milling method [10]. The objective of this study was to develop a nanosuspension of Irbesartan using the solvent evaporation and emulsion solvent diffusion methods. Irb, an Angiotensin II receptor blocker, is primarily used to treat hypertension [11]. As a BCS class II drug, Irb has a very low aqueous solubility of 5.9×10^{-2} mg/ml, making it practically insoluble [11]. Therefore, the formulation of Irb into a nanosuspension aims to enhance its solubility and improve its dissolution rate using various ratios of stabilizers such as PVP K30, Poloxamer 188, HPMC E15, and Tween 80, using organic solvents like ethanol, dichloromethane (DCM) and DMSO (Dimethyl sulfoxide).

The emulsion solvent diffusion method is straightforward and requires relatively simple equipment, making it suitable for both laboratory and industrial-scale production. Unlike high-pressure homogenization or other mechanical methods, this technique does not rely on intensive energy inputs, reducing operational costs. This method does not require high-cost or specialized machinery, making it more accessible. It allows the use of both organic and aqueous phases, offering flexibility to optimize

for specific drugs and solvents. By producing drug particles in the nanometer range, this method significantly increases the surface area, leading to enhanced dissolution rates and bioavailability for poorly water-soluble drugs.

Materials and methods:

Materials:
Irbesartan was a kind gift sample from Aurobindo Pharma limited Hyderabad, India. PVP K30 and Poloxamer 188 was procured from SD Fine chem limited, Hyderabad, India. HPMC E15 was a gift sample from Aurigen pharmaceutical services Limited, Hyderabad, India. Tween 80 was from Sisco Research Laboratories Pvt. Ltd. Hyderabad.

Method:
Method development for analysis of Irbesartan:
1) Determination of λmax of Irbesartan in 0.1N HCl:
10µg/ml solution of Irb was prepared in 0.1N HCl and analysed using a UV double beam spectrophotometer (®T60 UV VIS Spectrophotometer), in the range of 200-400nm to determine its λmax. [11]

2) Construction of calibration curve of Irbesartan in 0.1N HCl:
From a 100µg/ml solution, various samples in the range of 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6millilitre were transferred into a 10millilitre volumetric flask and the volume was made up to 10ml by using 0.1N HCl to prepare 1,2,3,4,5 and 6µg/ml solutions. The absorbance of these solutions was measured using UV spectrophotometer at 209nm and the process was repeated three times. The standard graph was plotted with concentration on x-axis and absorbance on Y-axis. [11]

PREFORMULATION STUDIES:

1) Determination of melting point of Irbesartan:
The melting point of a sample was determined using capillary melting point tubes. [11]

2) Fourier transform infrared spectroscopy (FTIR):
Using an Aligent Cary 630 FTIR, the drug Irbesartan and its nanosuspension were subjected to FTIR analysis. For the most effective contact between the sample and the crystal, the sample was put onto the ATR crystal and forced down using a swivel press. In contrast, a little drop of nanosuspension was applied to the ATR crystal and a measurement was made in order to assess the nanosuspension composition. Once the measurement is finished, the crystal is cleaned using an appropriate solvent. The primary peaks in the spectrum were examined after it was captured in the frequency range of 4000-650 cm^{-1}. [11,13, 15]

APPROACHES OF PREPARATION OF NANOSUSPENSIONS:
The nanosuspensions were prepared using Solvent evaporation method and Emulsion solvent diffusion technique.

Solvent Evaporation method:
75mg of Irbesartan was weighed and dissolved in organic solvent (Ethanol/ DCM) to prepare the organic phase (table 1 (a) & (b)). The aqueous phase was prepared by adding stabilizers (PVP K30/ Poloxamer 188/ HPMC E15 and tween 80) in water. The organic phase was then added drop wise into aqueous phase using a syringe and stirred for 2 hours using an over-head stirrer at 3000 rpm. The solution was then subjected to homogenization using probe sonicator for another hour at 3000rpm to evaporate the solvent. [16]

Table 1(a): Formulation of nanosuspension by solvent evaporation method

Ingredients	E1	E2	E3	E4	E5	E6	E7	E8	E9
Drug(mg)	75	75	75	75	75	75	75	75	75
PVP K30(mg)	75	100	125	-	-	-	-	-	-
Poloxamer 188 (mg)	-	-	-	75	100	125	-	-	-
HPMC E15 (mg)	-	-	-	-	-	-	75	100	125
Tween 80 (ml)	0.2	0.3	0.4	0.2	0.3	0.1	0.2	0.3	0.4
Ethanol (ml)	5	5	5	5	5	5	5	5	5
Water (ml)	40	40	40	40	40	40	40	40	40

Table 1(b): Formulation of nanosuspension by solvent evaporation method

Ingredients	E10	E11	E12	E13	E14	M1	M2	M3
Drug(mg)	75	75	75	75	75	75	75	75
PVP K30(mg)	-	-	-	-	-	50	75	100
Poloxamer 188 (mg)	125	150	150	75	75	50	-	-
HPMC E15 (mg)	-	-	-	75	75	-	-	-
Tween 80 (ml)	0.8	0.8	0.4	0.6	-	0.4	0.2	0.3
Ethanol (ml)	5	5	5	5	5	-	-	-
Dichloromethane(ml)	-	-	-	-	-	10	10	10
Water (ml)	40	40	40	40	40	40	40	40

Emulsion solvent diffusion method:

Drug (75mg) was dissolved in organic solvent (DMSO/ DCM), to give the organic phase. The aqueous phase (40ml) was prepared by adding stabilizers (PVP K30/ Poloxamer 188/ HPMC E15 and tween 80) in water (table 2). The organic phase was then added drop wise using a syringe and stirred for 2 hours using an over-head stirrer at 3000rpm. The suspension thus formed was then homogenized further at 3000rpm using probe sonicator for an additional hour. Finally, the suspension was diluted with 40ml double distilled water to induce diffusion of organic solvent into continuous phase. [17]

Table 2: Formulation of nanosuspension by emulsion solvent diffusion method

Ingredients	D1	D2	D3	D4	D5	M4	M5
Drug(mg)	75	75	75	75	75	75	75
PVP K30(mg)	75	100	125	-	-	75	150
Poloxamer 188 (mg)	-	-	-	75	-	75	150
HPMC E15 (mg)	-	-	-	-	75	-	-
Tween 80 (ml)	0.4	0.6	0.6	0.4	0.4	0.6	0.6
DMSO (ml)	5	5	5	5	5	-	-
Dichloromethane(ml)	-	-	-	-	-	10	10
Water (ml)	80	80	80	80	80	80	80

EVALUATION OF NANOSUSPENSION:

1) *Entrapment efficiency:*

Entrapment efficiency was determined to see the amount of drug entrapped in the suspension. The absorbance of the supernatant solution was measured at 209nm using a UV spectrophotometer to determine the amount of medication that had not been integrated. [17]

$$\text{Entrapment Efficiency (\%)} = \frac{(\text{W initial drug} - \text{W free drug})}{\text{W initial drug}} * 100$$

2) *In-vitro release studies:*

Drug release studies were performed by dialysis bag method. The dialysis bag was soaked overnight in 0.1N HCl. It is then tied into a bag and 4ml of nanosuspension was taken into the bag. The dialysis bag was immersed in the dissolution vessel containing 500ml of 0.1N HCl using USP type- I dissolution apparatus. To maintain sink conditions, an aliquot of 3millilitre samples was taken out at regular intervals. At the same time, new buffer was added to the medium. After calibrating the UV spectrophotometer with the appropriate blank, the obtained samples were analysed at 209 nm.

3) *Total drug content:*

A 0.5 ml aliquot of the prepared nanosuspension was dried by evaporation. A 0.45 µm filter was used to filter the residue after it had been dissolved in methanol. Using a UV spectrophotometer absorbance was measured and the total amount of drug was calculated. [13,17]

$$\text{Total Drug Content} = \frac{(\text{Total volume of nanosuspension} \times \text{amount of drug in aliquot})}{\text{Volume of aliquot}}$$

CHARACTERISATION OF IRBESARTAN NANOSUSPENSION:

1) Particle size and Zeta potential:

Dynamic Light Scattering (DLS) technique was used to measure the particle size of the D2 formulation at 25.1°C and a scattering angle of 173°. Zetasizer (HORIBA SZ-100) was used to assess the zeta potential of nanosuspension. Zeta potential measurements are frequently used to evaluate the stability of suspensions because they provide a very strong indicator of the strength of the interactions between particles. In aqueous media, the majority of particles have an electric charge. [13,14,15]

2) Surface Morphology of Irbesartan nanosuspension:

The nanosuspension formulation's form and surface morphology were investigated using a Hitachi S-3800N® scanning electron microscope (SEM). SEM was used to see the nanoparticles' three-dimensional pictures. A single drop of nanosuspension was placed on a transparent glass-covered stub and let to air dry. Sodium aurothiomalate was used to apply the gold coating, which was then examined at 10,000 SEM magnification. [13,14,15]

3) XRD:

To confirm the crystal form transition of Irbesartan after preparation of nanosuspension, the pure form of drug and the nanosuspension of irbesartan were analysed in *XRDML, with intended wavelength type of K-α1 radiation, generated at 30 mA and 45 kV. With Start Position [°2θ] 3.0179, End Position [°2θ] 39.9779, Step Size [°2θ] 0.0330 and Scan Step Time [s] 67.9450. [13,14, 15]

LITERATURE REVIEW:

Jaydeep patel et al (2011), formulated self-nanoemulsifying drug delivery system of Irbesartan. The aim of the investigation was to develop a self-nanoemulsifying drug delivery system (SNEDDS) to enhance the oral bioavailability of poorly water-soluble IRB. The solubility of IRB in various oils was determined to identify the oil phase of SNEDDS. Various surfactants and co-surfactants were screened for their ability to emulsify the selected oil. Pseudoternary phase diagrams were constructed to identify the efficient self-emulsifying region. The optimized SNEDDS formulation contained IRB (75 mg), Cremophor ® EL (43.33%), Carbitol® (21.67%) and Capryol® 90 (32%). SNEDDS was further evaluated for its percentage transmittance, emulsification time, drug content, phase separation, dilution, droplet size and zeta potential. The optimized formulation of IRB-loaded SNEDDS exhibited complete in vitro drug release in 15 min as compared with the plain drug, which had a limited dissolutionrate. It was also compared with the pure drug solution by oral administration in male Wister rats. The in vivo study exhibited a 7.5-fold increase in the oral bioavailability of IRB from SNEDDS compared with the pure drug solution. These results suggest the potential use of SNEDDS to improve dissolution and oral bioavailability of poorly water-soluble IRB.

Sai Kishore Meruva et al (2019), formulated Irbesartan nanocrystalline suspension *via* media milling. Nanocrystalline suspensions offer a promising approach to improve the dissolution rateof BCS Class II/IV drugs and hence oral bioavailability. Irbesartan (crystalline Form B), a poorly soluble drug substance was chosen as a model compound for the study. A Design of Experiment approach was utilized to understand the impact of formulation variables on particle size reduction *via* media milling. Drug concentration and type of stabilizer were found to be significant in particle size reduction. Optimized Irbesartan nanocrystalline suspension (*i.e.* at 10% w/w with 1% w/w poloxamer 407) showed superior *in vitro* dissolution profile compared to unmilled suspension. Optimized Irbesartan nanocrystalline suspension was converted into dried powders either by bead layering (with microcrystalline cellulose) or by spray granulation (either with mannitol or microcrystalline cellulose). DSC and PXRD studies revealed that Irbesartan remained crystalline post drying. Microcrystalline cellulose beads layered with Irbesartan nanocrystals showed about 65% drug dissolution within the first 10 min of dissolution study. Mannitol granules containing Irbesartan nanocrystals were fast dissolving (*i.e.* >90% drug dissolution within 10 min) compared to microcrystalline cellulose granules(*i.e.* approx. 46% drug dissolution within 10 min). Irbesartan nanocrystalline suspension had the fastest dissolution rates (*i.e.* >90% drug dissolution in two minutes) followed by mannitol- based granules containing dried Irbesartan nanocrystals *(i.e.* >90% drug dissolution in ten minutes).

Rikisha Boghra et al (2011), formulated and evaluated Liquisolid tablets of irbesartan. The liquisolid tablets of Irbesartan were prepared by using various ratio of carrier (Avicel PH 102)to coating (Cab-O-Sil M5) material using PEG 400 as non-volatile solvent. They did a study to improve the dissolution and there by availability of Irbesartan a practically insoluble drug by liquisolid Compact technique. The prepared liquisolid tablets were evaluated for hardness, friability, disintegration time. The dissolution profile of Irbesartan tablets were determines according to USP method and compared to that of a direct compressible tablet. The formulated liquisolid system of Irbesartan exhibited acceptable flowability and compressibility. All the formula of liquisolid tablets showed more than 90% release within 60 minutes. Technique of Liquisolid tablet of Irbesartan which can be scaled-up industrially is promising approach for enhancing solubility and dissolution rate.

This was due to an increase in wetting properties and surface of drug available for dissolution.

Rusul M. Alwan et al (2021), prepared and optimized Selexipag nanosuspensions (SLPNS) to enhance the saturation solubility and in vitro dissolution rate. Selexipag (SLP), is an orally selective long-acting prostacyclin receptor agonist indicated for pulmonary arterial hypertension treatment. It is practically insoluble in water (class II, according to BCS). The solvent antisolvent precipitation method was used for the production of NS, andthe effect of formulation parameters (stabilizer type, drug: stabilizer ratio, and use of co- stabilizer) and process parameter (stirring speed) on the particle size (P.S) and polydispersity index (PDI) were studied. The result revealed that the P.S of all prepared SLPNS formulation was in the nanometer range, except for the formulas that stabilized by Poloxamer. The optimal SLPNS (F15), which is stabilized by Soluplus® (SLP: stabilizer ratio 1:2) and prepared at a stirring speed of 1000 rpm, showed the smallest P.S and appropriate PDI, which are 47 nm and 0.073. The formula F5 exhibits 136 folds, an increase in the saturation solubility, and an enhancement in the dissolution rate in phosphate buffer pH 6.8 (100% drug release during 60 min) compared to the pure drug. This result indicates that SLPNS is an efficient way for improving the saturation solubility and the dissolution rate of SLP.

William wei lim chin et al (2014), described how Particle size reduction can be used for enhancing the dissolution of poorly water-soluble drugs in order to enhance bioavailability. In nanosuspensions, the particle size of the drug is reduced to nanometer size. Nanosuspensions after downstream processing into drug products have successfully shown its impact on formulation design, the augmentation of product life cycle, patent life, and therapeutic efficacy.Formulation considerations for the nanosuspension formulation, its processing into a solid form, and aspects of material characterization are discussed. Technology assessments and feasibility of upstream processes for nanoparticle creation, and subsequently transformation into a drug product via the downstream processes have been reviewed. This paper aims to bridge formulation and process considerations along with patent reviews and may provide further insight into understanding the science and the white space. An analysis of current patentoutlook and future trends is described to fully understand the limitations and opportunities in intellectual property generation.

Prasanna lakshmi* et al, (2010), described how Solubility is the crucial factor for drug effectiveness, independence of the route of administration. Large proportions of newly discovered drugs are water insoluble, and therefore poorly bioavailable contributing to deserted development effort. These so-called 'Brickellia' candidates can now be delivered by formulating them into nanosuspension. Nanosuspension technology solved the problem of drugs which are poorly aqueous soluble and less bioavailability. Stability and bioavailability of the drugs can be improved by the Nanosuspension technology. Preparation of nanosuspension is simple and applicable to all drugs which are aqueous insoluble. Nanosuspensions are prepared by using wet mill, high pressure homogenizer, emulsion-solvent evaporation, melt emulsification method and super critical fluid techniques. Nanosuspensions can be delivered by oral, parenteral, pulmonary and ocular routes. Nanosuspensions can also be used for targeted drug delivery when incorporated in the ocular inserts and mucoadhesive hydrogels.

Shivraj Popat Jadhav* et al, (2023), described that the formulation development of a drug is majorly based on the solubility of drugs. Nearly 40% of newly developed drugs show poor water solubility. Several approaches are used for the increasing solubility of such drugs for formulation development. Nanosuspension is one such attractive tactic which can be used for the enhancement of solubility, stability as well as bioavailability of such drugs. Nanosuspension can be defined as a

two-phasic dosage form where solid drug particles of size less than 1 μm are dispersed in an aqueous phase with the help of a stabilizer. Particle size reduction in the nanoscale causes an increase in the surface area leading to an increase in dissolution rate and ultimately bioavailability is increased. A variety of excipients are used for the formulation of nanosuspension. Stabilizers, surfactants, co-surfactants, natural as well as organic solvents, buffers, salts, polyols, osmogents, and cryoprotectants are used in the formulation of nanosuspension. Various methods used in the formulation of nanosuspension can be classified into top-down and bottom-up approaches. Wet milling, high-pressure homogenization, anti-solvent techniques, melt emulsification, supercritical fluid extraction and ultrasonic homogenization are some of the methods for the preparation of nanosuspension. These methods are easy and appropriate for all poor water-soluble drugs. Nanosuspensions technology can be applied for the preparation of oral, pulmonary, injectable, ocular as well as targeted drug delivery. This review emphasizes various advantages and disadvantages of nanosuspension, its methods of preparation, formulation approaches, evaluation parameters, pharmaceutical applications, marketed products and patents of nanosuspension.

Yingying Ma et al, (2023), described that the drug nanosuspensions is a universal formulation approach for improved drug delivery of hydrophobic drugs and one the most promising approaches for increasing the biopharmaceutical performance of poorly water-soluble drug substances, especially for nature products. This review aimed to summarize the nanosuspensions preparation approaches and the main technological difficulties encountered in nanosuspensions development, such as guidelines for stabilizers screening, in vivo fate of the intravenously administrated nanosuspensions, and how to realize the intravenously target delivery was reviewed. Furthermore, challenges of nanosuspensions for the nature products delivery also was discussed and commented. Therefore, it hoped to provide reference and assistance for the nanosuspensions production, stabilizers usage, and predictability of in vivo fate and controllability of targeting delivery of the nature products nanosuspensions.

Vidyadhara Suryadevara et al (2016), developed fast dissolving tablets of Irbesartan in order to enhance the rate of its dissolution. The objective of the investigation was to enhance the solubility and dissolution rate of poorly soluble drug irbesartan by preparing it as solid dispersions and formulating it as fast-dissolving tablets (FDTs) using various excipients because Irbesartan is poorly soluble in water, and this low aqueous solubility in addition to its poor wettability leads to poor bioavailability of the drug. Solid dispersions were prepared using Soluplus, PEG 6000, and Kollidon as carriers. The dispersions were prepared using the solvent evaporation and kneading methods in a 1 : 1 ratio of drug and carrier. The aqueous solubility ofirbesartan in solid dispersions was improved by the presence of polymer Soluplus when compared with other carriers. Solid state characterization indicated that irbesartan was present as amorphous material in the formulation with carrier. This was due to efficient entrapment of the drug in polymer matrix. Thus, the solid dispersion prepared with Soluplus would be useful for delivering poorly soluble irbesartan with enhanced solubility and dissolution rate. Furthermore, the solid dispersions that were formulated as FDTs using superdisintegrants showed faster drug release with increased dissolution rate.

Swetha Konda et al (2013) prepared Mucoadhesive Microspheres of Irbesartan by orifice Ionic Gelation Technique employing polymers like Hydroxy Propyl Methyl Cellulose, Carbopol along with Sodium alginate. The Microspheres prepared were discrete, spherical and free flowing. Microspheres were evaluated for Flow properties, Particle size, Percentage yield, Drug entrapment efficiency, Percentage moisture loss, Swelling index, Loose surface crystal, invitro wash-off test, in vitro drug release and drug release kinetics. The drug polymer interactionstudy was conducted by FT-IR and results indicate that there was no interaction between Irbesartan and polymers. The Percentage yield, Drug entrapment efficiency Particle size, swelling index, Loose surface crystal and

Percentage moisture loss of best formulation, F6 was found to be 88.12%, 83.06 ± 0.43%, 7.65 ± 0.47µm, 194 ± 3.65%, 22.32 ± 0.34% and 7.06 ± 0.45% respectively. The in vitro wash-off test indicated that the microspheres had good mucoadhesive properties. The in-vitro dissolution studies showed that Irbesartan MucoadhesiveMicrospheres formulation F6 showed better sustained effect (94.97%) over a period of 8 hours than other formulations. Drug release was diffusion controlled and followed first order kinetics.Hence, prepared Mucoadhesive Microspheres may be an effective strategy for the development of easy, reproducible and cost-effective method for safe and effective oral drug therapy.

RESULTS AND DISCUSSION:

Analytical method development for Irbesartan:
Absorption maximum of Irbesartan was determined to find a suitable wavelength for analysing the drug sample.

1) Determination of λmax in 0.1N HCl:
10 µg/ml solution of irbesartan was scanned using UV Vis spectrophotometer in the range of 200-400nm. Irbesartan showed maximum absorbance at 209nm as shown in fig 1(a). This is where the drug absorbs maximum light. To check if any of the excipients had any interference, a placebo was also scanned and there was no interaction (fig 1(b)).

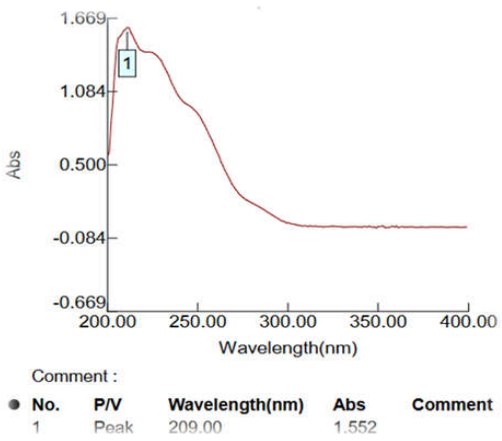

figure 1(a)

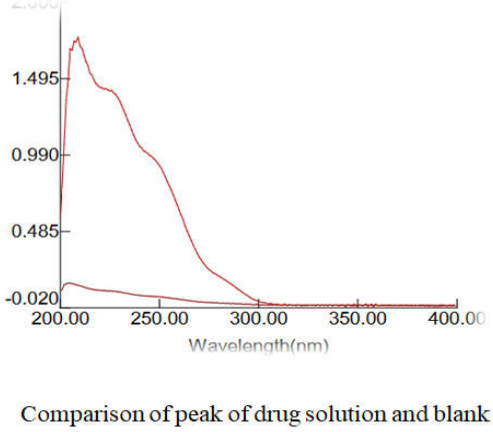

figure 1(b)

Figure 1: λmax of Irbesartan in 0.1N HCl

2) Construction of calibration curve of Irbesartan in 0.1N HCl:
The standard graph was plotted to determine the concentration of unknown sample in 0.1N HCl with concentration on the x-axis and absorbance on the y-axis as shown in fig 2.

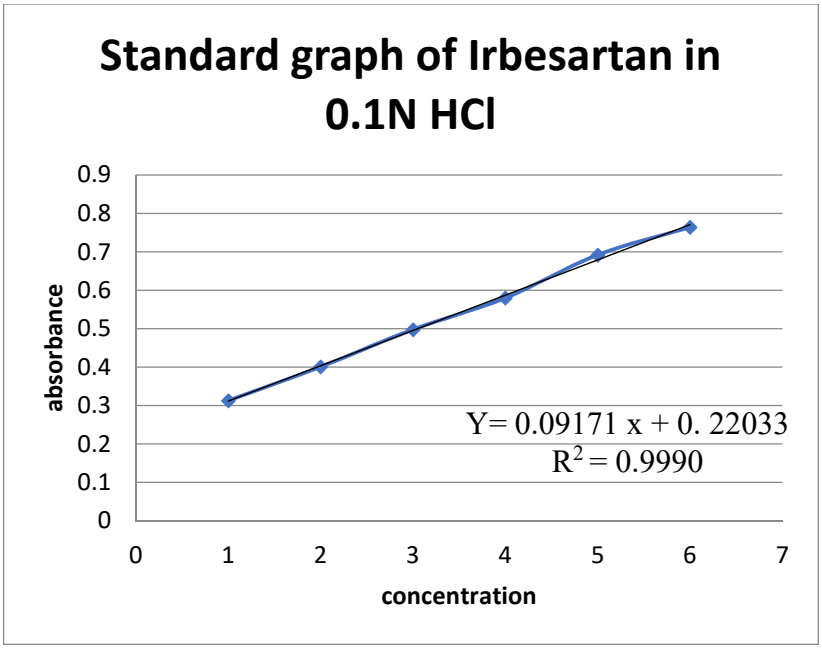

Figure 2: Standard graph of Irbesartan in 0.1N HCl

As seen in Fig 2, the equation for the standard graph was found to be y= 0.09171x +0.2203 with an R^2 value of 0.9990. Therefore, the graph is linear in the range of 1 to 6µg/ml.

PREFORMULATION STUDIES:

1) Determination of melting point of Irbesartan:
Melting point was performed by capillary tube method to determine the purity of the sample. The melting point was found to be 180^0 C, which is close to reported value i.e. 180-181 ^{0} C

2) FTIR:
The drug's stability and the presence of any intermolecular interactions were determined using Fourier transform Infrared spectroscopy. The Irbesartan and Irbesartan nanosuspension FTIR spectra are shown in fig 3. The results show that there was no interaction between Irb and excipients. The absorption bands displayed by Irbesartan and its nanosuspension are shown by their wave numbers in Table 3.

Table 3: Wave numbers of peaks obtained for drug and Irbesartan nanosuspension

Functional groups	Wave number (cm^{-1}) of peaks obtained for drug	Wave number (cm^{-1}) of peaks obtained for nanosuspension
C=O	1684	1636
C-N	1237, 1261	1239
C-H (aromatic)	2931, 2957	2927
C=N	1612	1636
C-H (aliphatic)	1407, 1436	1412
C=C	2364, 2517	-
N=N	1522, 1599	1500
N-N	2364	-
O-H	-	3365

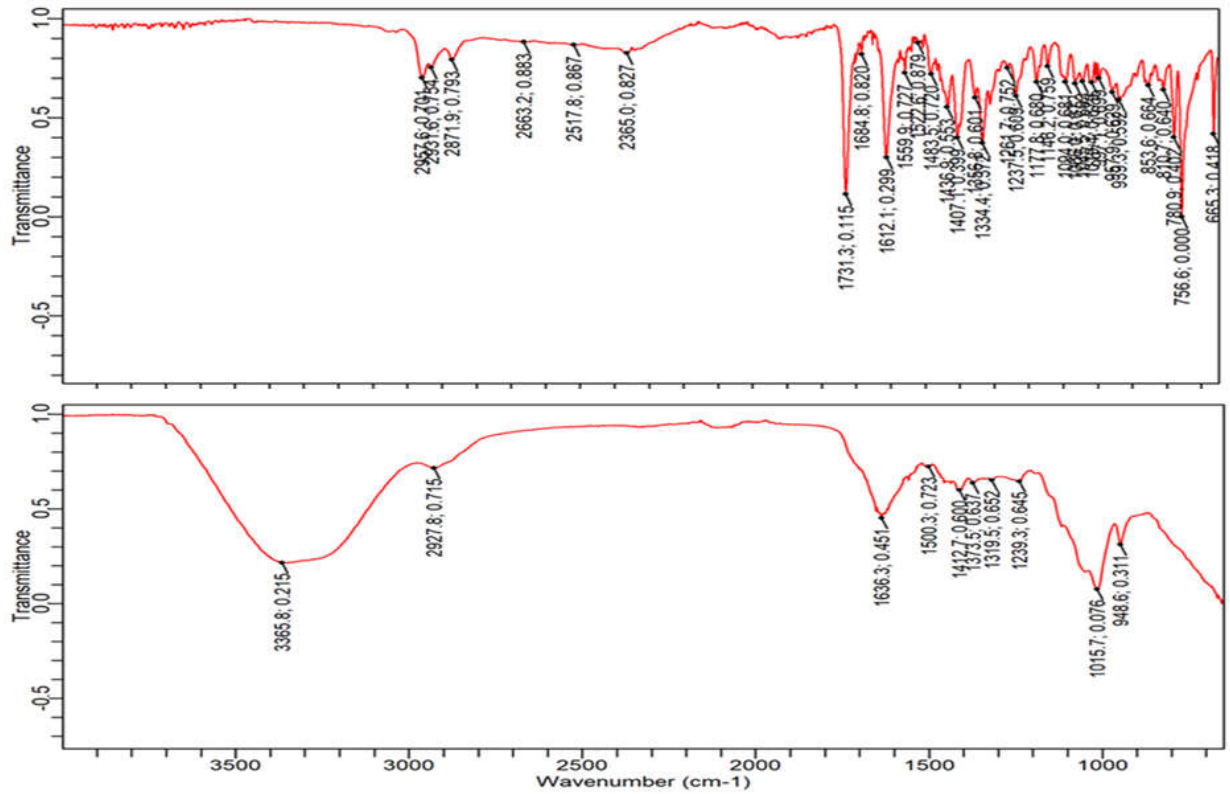

Figure 3: FTIR Spectrum of Irbesartan and Irbesartan nanosuspension

Preparation of Nanosuspension:

Irbesartan nanosuspensions were prepared by solvent evaporation method and emulsion solvent diffusion method. There was a delay in drug release for nanosuspension prepared by solvent evaporation method. Further, the method also has additional disadvantages like re-aggregation of particles, limited solubility of drug; stability etc. To overcome these disadvantages emulsion solvent diffusion technique was adopted. In addition, emulsion solvent diffusion method has following advantages like controlled particle size, high drug loading efficiency, heat- free process.

EVALUATION OF NANOSUSPENSION:

1) Entrapment efficiency:
Highest entrapment was seen for formulation D2 which is 99.27% and lowest for formulation M4 which is 83.39%.

2) In-vitro drug release studies:
Formulations were prepared by solvent evaporation method using Ethanol (E1 to E14) and DCM (M1 to M3) as organic solvents. With formulations prepared using ethanol, maximum drug release was obtained with E8, i.e. 74.16% in 5 hours. With formulations prepared using Dichloromethane (DCM), the maximum drug release was obtained with M2, i.e. 35.64% after 75 minutes, which is not desirable. Thus, we tried formulating the nanosuspension by emulsion solvent diffusion method using Dimethyl sulfoxide (DMSO) (D1 to D5) and DCM (M4, M5) as the organic solvent. With DCM, the maximum drug release was about 36% in 75 mins but With DMSO, that is with formulation D2, the drug release

was 94% within the first 10 minutes, which is desirable. Hence, this was selected as the best formulation.
The nanosuspensions prepared by solvent evaporation method using Ethanol and DCM as organic solvents did not give the desired drug release, probably because the solvent did not evaporate completely. The other possible reasons could be, recrystallization of the drug, after solvent evaporation, the drug may have recrystallized or formed aggregates, reducing its solubility and, consequently, its release from the suspension. Inadequate Wetting of the Drug, or incomplete removal of the organic solvent (ethanol/ DCM) could affect the drug's properties and solubility.

Nanosuspensions prepared using DCM, by emulsion solvent diffusion method, did not give desired drug release, and the possible reasons for this could be- limited solubility of the drug in the solvent, DCM. DCM is more volatile, which can lead to rapid evaporation during emulsification. This may result in premature precipitation of the drug.

On the other hand, nanosuspensions prepared by emulsion solvent diffusion method using DMSO as solvent provided the desirable drug release. The reason could be because of the better Solubility of Drug in DMSO (approximately 14mg/ml). Other possible reasons could be, Lower Vapour Pressure and Slower Evaporation rate of DMSO. DMSO has a higher boiling point (189°C) compared to ethanol (78°C) or DCM (39.6°C), which means it evaporates more slowly. This slow evaporation rate allows for a more controlled and gradual precipitation of drug nanoparticles, promoting the formation of smaller, more uniformly sized particles. A graph depicting the release profiles of formulations D1 to D5 is shown below in fig 4(a).

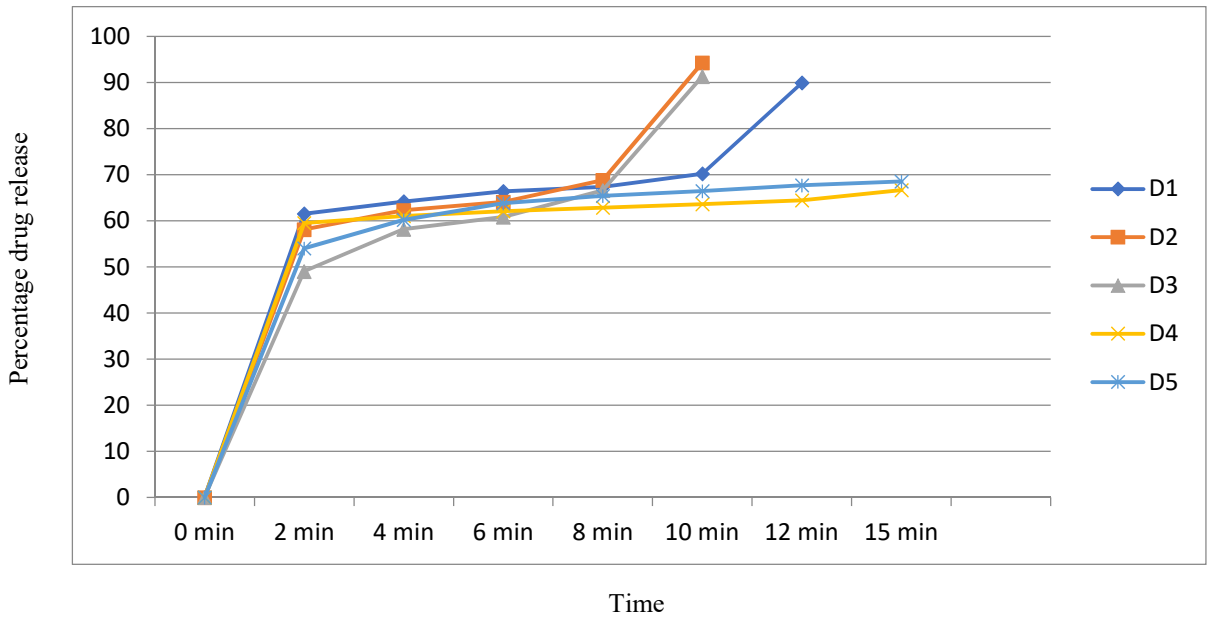

Figure 4(a):A graph depicting the release profiles of formulations D1 to D5

Formulation D1 showed 89.94% release in 12mins, D2 showed 94.29% release in 10mins, D3 showed 91.3% release in 12mins, D4 showed 66.67% release after 15 mins, D5 showed 68.56% release after 15 mins. That is why D2 was selected as best formulation.

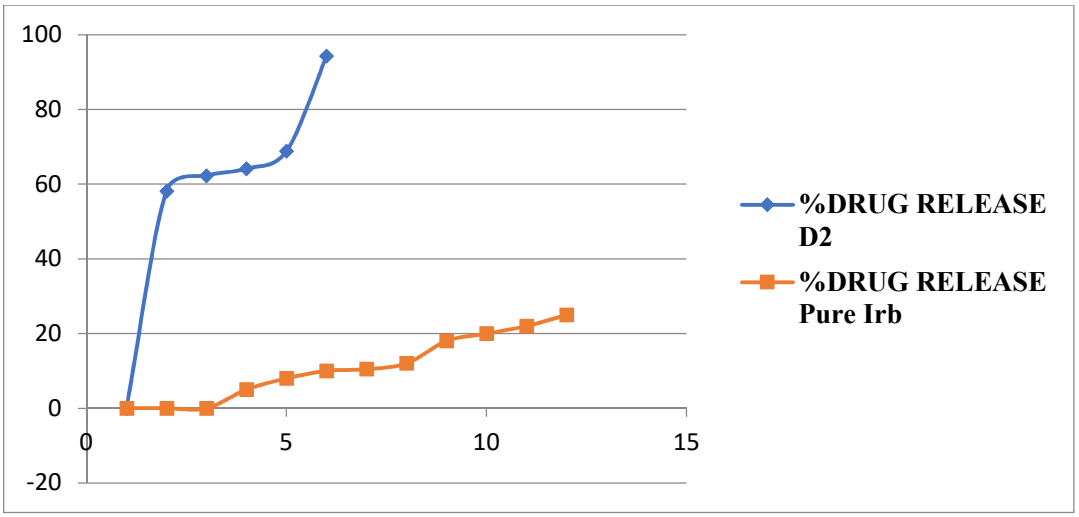

Figure 4(b): Comparision of *In-vitro* release profiles of Pure Irb and D2

As seen in fig 4(b), The drug release profile of best selected formulation was compared to the plain drug. Pure drug showed 25% release in 75 minutes, where as formulation D2 showed 94.29% release in 10 minutes only.

3) Total drug content:
Total drug content analysis was performed for the best selected formulation D2, and it showed 95.56% drug content.

CHARACTERISATION OF IRBESARTAN NANOSUSPENSION:

1) Particle size and Zeta potential:
It was found that the average particle size was 266.5 nm. One crucial factor that affects the distribution of sample particle sizes is the polydispersity index. The degree of particle size heterogeneity in a sample is measured by the PDI. PDI for the nanosuspension was 0.448, indicating slight variation in the particle size distribution. The D2 formulation's particle size distribution is neither significantly polydisperse nor monodisperse. Therefore, it was verified that formulation D2 has moderate amounts of both tiny and big particles. The particle size of nanosuspension is shown in fig 5.

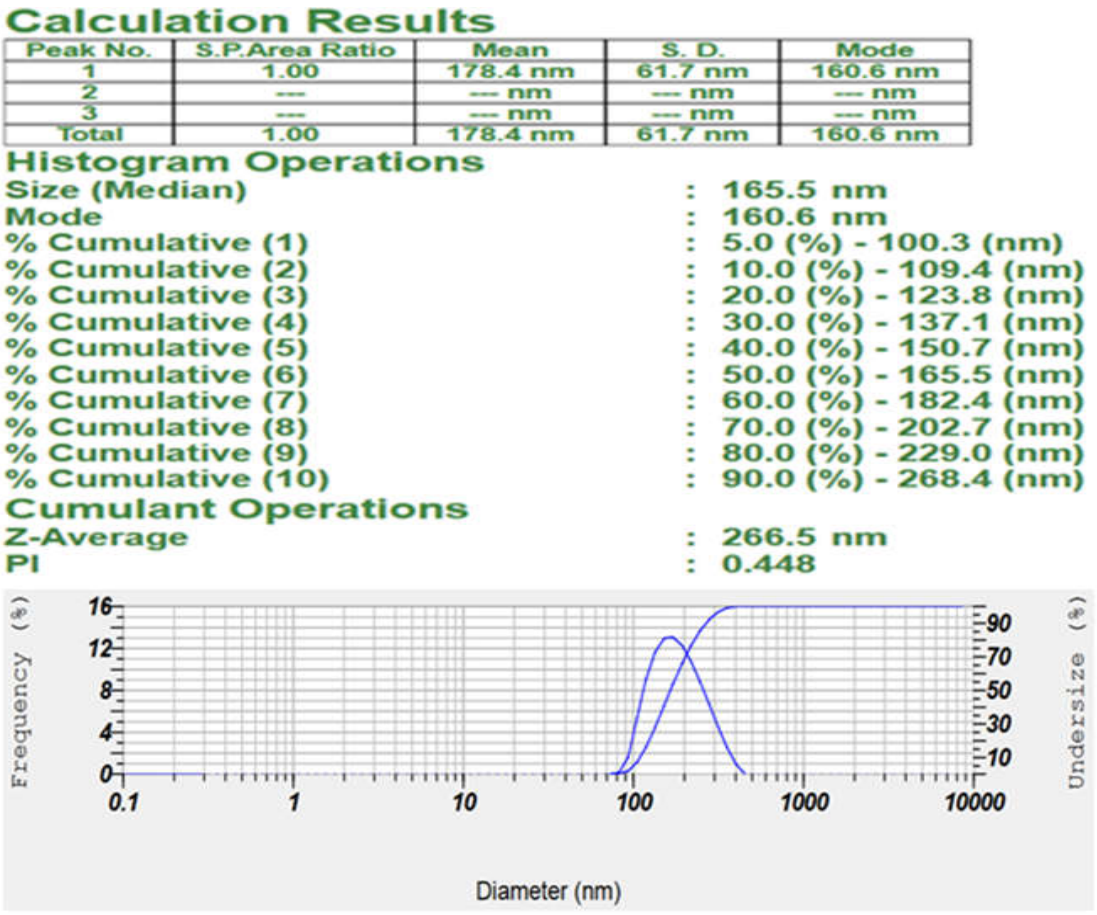

Figure 5: Particle size of Irbesartan nanosuspension

Zeta potential:

The zeta potential value of the D2 formulation is given in Fig 6. The zeta potential values of the D2 formulation obtained were negative (-32.2 mV). The surface of particles was negatively charged. The zeta potential value indicates that the particles do not have the tendency to re-aggregate and the suspension is relatively stable. This is also confirmed in Scanning electron microscopy, where we can see particles existing as individual entities.

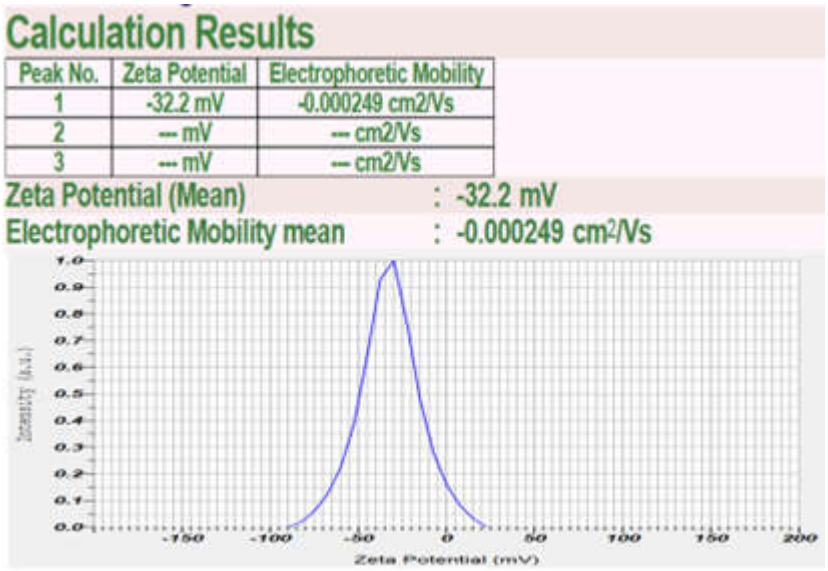

Figure 6: Zeta potential of Irbesartan nanosuspension

2) Surface morphology:

As seen in the fig 7, the particle size ranged from 54.69 nm to 359.3 nm. The particles were uniformly scattered, there was no aggregation, and the particles existed as individual entities and were discrete.

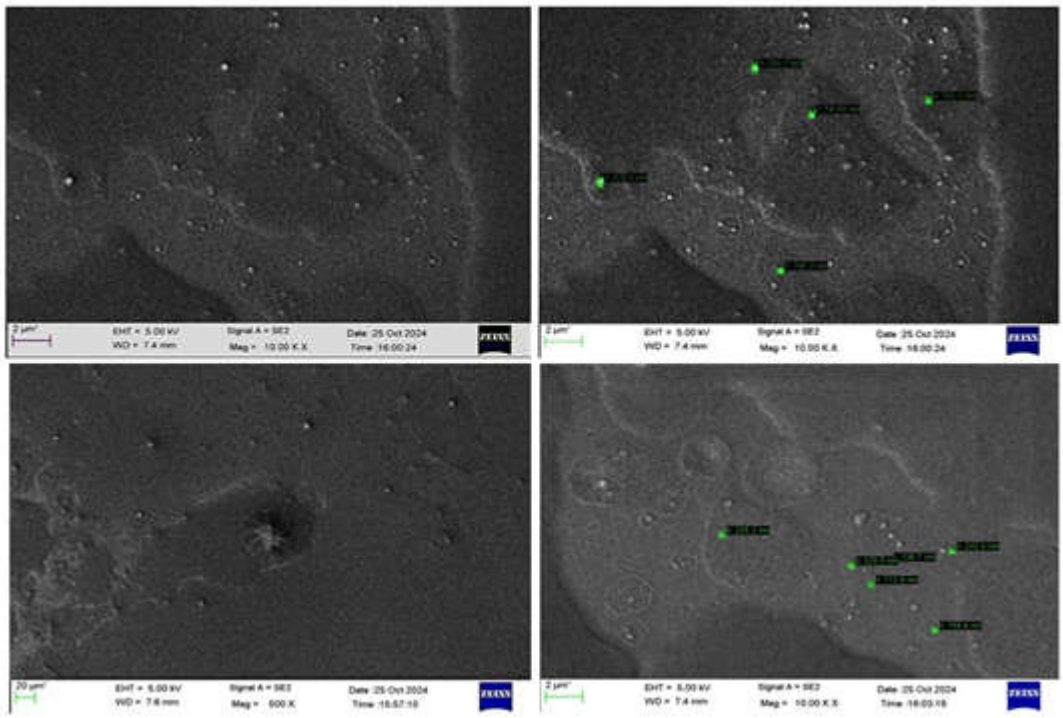

Figure 7: SEM images of Irbesartan nanosuspension

3) XRD:

XRD was used to validate the crystalline state changes of irbesartan nanosuspensions. Pure irbesartan and prepared nanosuspension of optimised formulation were the samples utilized for measurement. The distinctive crystalline peaks of irbesartan were found at 12.2102°, 19.1593°, 19.7714°, 20.304°, 20.847°, and 22.8994°, in the pure irbesartan patterns, as seen in fig 8.

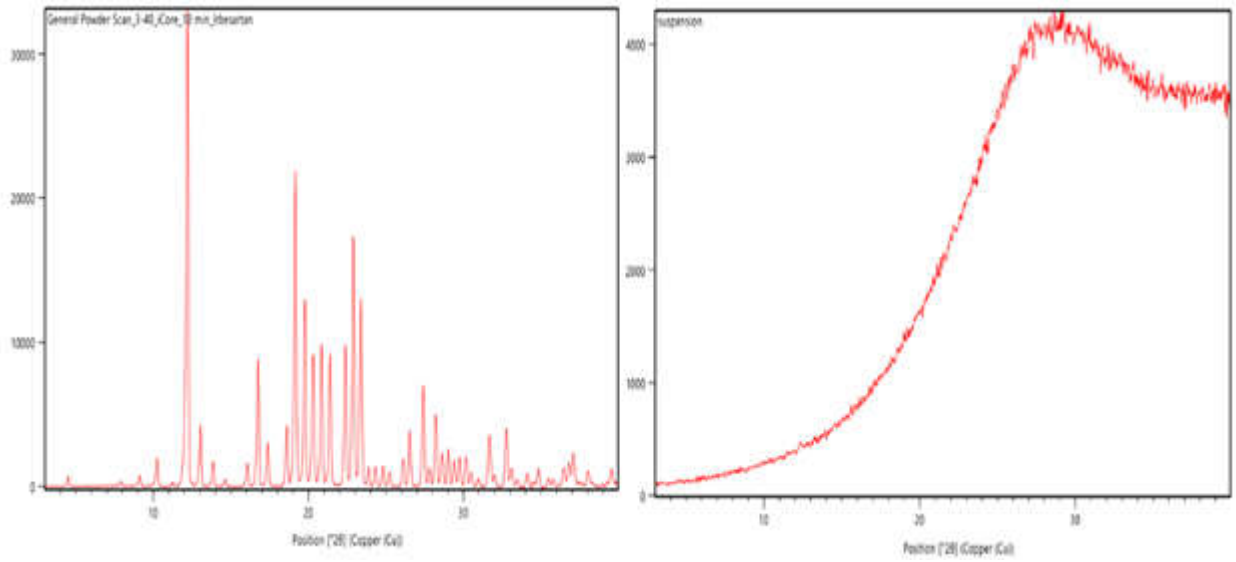

Figure 8: XRD of irbesartan and Irbesartan Nanosuspension

However, the profile of the Irbesartan nanosuspension, as shown in fig 8. did not show a clear peak of pure Irbesartan, suggesting that the crystalline structure of Irbesartan was lost in Nanosuspension, indicating its conversion into amorphous form.

CONCLUSION:

The Emulsion solvent diffusion method was successfully employed to produce stable Irbesartan nanosuspension. Nanosuspension with an average particle size of 266.5nm was obtained. There was a substantial change in the crystalline nature of the drug after formulating into nanosuspension. Irbesartan showed improved dissolution rate after formulating into nanosuspension, which is evident from the acquired results. The zeta potential value indicates that the nanosuspension is stable, which is further confirmed from the SEM images, which also shows that the particles are uniformly distributed and exist as individual entities.

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