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# FORMULATION AND CHARACTERISATION OF VARIOUS FORM OF CRYSTALS OF CEFUROXIME AXETIL AND AZTREONAM.

**Balasubramanian V[1]*, Senthil Kumar K L[2], Vijaya K[3]**

[*1]Professor, Department of Pharmaceutics, Jainee College of Pharmacy, Dindugal, Tamilnadu. Affiliated The TN Dr MGR Medical University, Chennai, Tamilnadu, India.
[2]Principal, Sri Vijay Vidyalaya College of Pharmacy, Dharmapuri, Tamilnadu. Affiliated The TN Dr MGR Medical University, Chennai, Tamilnadu, India.
[3]Dean, Ultra College of Pharmacy, Madurai, Tamilnadu. Affiliated The TN Dr MGR Medical University, Chennai, Tamilnadu, India.

**ABSTRACT**

In solid dosage forms, the first opportunity for a change in crystallinity will be in the blending and milling of drug with excipient to produce a homogeneous blend. Since most of the dosage forms are solids such capsules/tablets the focus will be on the processing parameters that are operative in the production of solid dosage forms. Solution dosage forms are generally independent of the polymorphic problems. If there is a less soluble form, it will appear on temperature cycling stability testing. In solid dosage forms, the first opportunity for a change in crystallinity will be in the blending and milling of drug with excipient to produce a homogeneous blend. In the light of the results of the present study, it is concluded that the crystalline forms of the selected drugs Cefuroxime axetil and aztreonam prepared by ammonia diffusion method did not undergo any phase transition by either formulation variables or manufacturing processes as shown by no changes in their stability and essential physico-chemical properties dependent on crystalline behavior.

**KEY WORDS**: Crystals, Cefuroxime axetil, Aztreonam, X-Ray, FT-IR spectra

## INTRODUCTION

For a product to possess the desired physicochemical properties, be convenient and cheap to manufacture, be easy to handle, be stable, meet regulatory requirements, be patentable, or not infringe upon existing patents, it is imperative to manufacture and develop the optimal solid dosage form. Obviously, the first step in selecting the optimal form is to identify all relevant solid forms that a new compound can exist in. The probability that a compound can exist in several solid forms is probably close to 100%, considering that 56-87% of all small organic molecules can form solvates and polymorphs alone [1]. A solid substance can be classified as being crystalline, non-crystalline, or a mixture of the two forms. In crystalline materials, the molecular or atomic species are ordered in a three- dimensional array, called a lattice, within the solid particles [2]. Non-crystalline solids sometimes are referred to as glasses or amorphous solids when repetitive order is non-existent in all three dimensions. Many compounds are capable of crystallizing in more than one type of crystal lattice. At any particular temperature and pressure, only one crystalline form (polymorph) is thermodynamically stable. Since the rate of phase transformation of a metastable polymorph to the stable one can be quite slow, it is not uncommon to find several polymorphs of crystalline pharmaceutical compounds existing under normal handling conditions [3]. In addition to exhibiting polymorphism, many compounds form crystalline solvates in which the solvent molecule is an integral part of the crystal structure. Examples for drugs already available in different polymorphs are Ciprofloxacin, Norfloxacin, Cefixime trihydrate –Cefotaxime etc. Polymorphs of a drug substance can have different apparent aqueous solubility and dissolution rate, when such differences are sufficiently large bioavailability is altered and it is often difficult to formulate a bioequivalent drug product using a different polymorph [4]. Aqueous solubility of drugs is traditionally determined using the equilibrium solubility method that involved suspending an excess amount of a solid drug in a selected aqueous medium. A polymorphism screening should at least contain the following elements.

Corresponding Author:- **Balasubramanian V** Email:-

As a reference, the starting material should be characterized by several methods, such as XRD (powder or single-crystal), DSC, TG-FTIR or TG-MS, DVS, Raman or IR spectroscopy, magic-angle spinning (MAS) NMR, solubility measurements, microscopy, and HPLC (purity). Antibiotic cefuroxime axetil (CA) is a second-generation cephalosporin antibiotic with low solubility and high permeability (BCS Class-II). There are 32% oral bioavailability and a short half-life for CA. Cefuroxime is hydrolysed by nonspecific esterase enzymes when CA is attached to the intestinal mucosa, and the highly polar carboxyl group is ionized in this environment. This condition occurs when a large amount of antibiotic enters the colon [5]. Minitablets with modified release muco-adhesion consist of multiple subunits, each containing a different dose of medication. Aztreonam is a totally synthetic monocyclic beta lactam belonging to a new class of antibiotics, the monobactams. In the present study, formulation of various type of crystals form of both Cefuroxime and aztreonam by means of various solvents and also characterised by FTIR and X-RAY Diffraction methods

**Methodology**

**Materials used**

Cefuroxime Axetil and Aztreonam were procured from Lupin Pharmaceuticals as gift sample.

**Preparation of Different Crystal Forms of Cefuroxime Axetil and Aztreonam**

**A. From Distilled Water**

The drug (0.5g) was dissolved in disti1led 50ml to check its solubility. To this solution, another weighed amount of Cefuroxime-Axetil (2.5g) was added and dissolved in distilled water (250ml), over water bath for 3 hours. The solution was filtered through Whitman filter paper and the filtrate was kept at room temperature to afford well defined crystals of Cefuroxime-Axetil. The crystals obtained were collected by filtration, dried under vacuum for 24 hours and stored in well closed container [6].

The aztreonam crystals obtained were collected by filtration, dried under vacuum for 24 hours and stored in well closed container [7].

**B. From Methanol**

The drug (0.5g) was dissolved in methanol (50ml) at its boiling point to check its solubility. To this solution, another weighed amount (2g) of Cefuroxime-Axetil was added and refluxed with methanol (230m1) for 90 minutes. The solution was filtered through Whitman filter paper and concentrated by recovery of the solvent to one third of its original volume and kept for crystallization at room temperature to afford well defined crystals of Cefuroxime-Axetil. The crystals obtained were collected by filtration, dried under vacuum for 24hours and stored in well container. The aztreonam crystals obtained were collected by filtration, dried under vacuum for 24 hours and stored in well closed container [8].

**C. From Ethanol**

The drug (0.5g) was dissolved in ethanol (45ml) at its boiling point to check its solubility. To this solution, another weighed amount (3.5g) of Cefuroxime-Axetil was added and refluxed with ethanol (280m1) for 2.5 hours. The solution was filtered through Whitman filter paper and the filtrate was concentrated by recovery of the solvent to one third of its original volume and kept at room temperature to afford well defined crystals of Cefuroxime-Axetil. The crystals obtained were collected by filtration, dried under vacuum at room temperature for 48 hours and stored in well closed container. The aztreonam crystals obtained were collected by filtration, dried under vacuum for 24 hours and stored in well closed container

**Preparation of Spherical Crystals**

**By Ammonia Diffusion Method**

Cefuroxime-Axetil was dissolved in aqueous ammonia (40m1, 20% w/v) and maintained at 40°C to avoid solubility problems. This solution was poured into a mixture of acetone (47. 0 ml) and dichloromethane (13.0ml) under agitation at 150-200 r.p.m using a magnetic stiffer. The system was thermally controlled at 18± 2°C throughout the process. After some time, solid particles started separating out. The agglomerated crystals were separated out by filtration. The crystals so obtained were dried under vacuum in a desiccator and finally kept in a dark and dry container, yield[77]. The aztreonam crystals obtained were collected by filtration, dried under vacuum for 24 hours and stored in well closed container [9].

**Evaluation and Characterisation of Cefuroxime-Axetil and aztreonam Crystals**

**Determination of Melting Point**

Determining the melting point of a compound is one way to test if the substance is pure. A pure substance generally has a melting range (the difference between the temperature where the sample starts to melt and the temperature where melting is complete) of one or two degrees. Impurities tend to depress and broaden the melting range so the purified sample should have a higher and smaller melting range than the original, impure sample.

**Solubility**

The solubility analysis was the quantitative determination of the purity of the substances through the application of precise solubility measurement. At a given temperature, a definite quantity of solvent. The resulting solution was saturated with respect particular substance, but the solution remains unsaturated with respect to other substances, even though such substances may be closely related in chemical structure and physical properties to the

substances[52]. To blend the series of separate solvent with increasing quantity of API with measured, fixed amount of the solvent, here temperature, pressure identical constant.

**Powder X-Ray Diffraction (PXRD) Analysis**

The X-ray powder diffraction pattern was reported on Bruker AXS D8 ADVANCE, equipped with Bragg-Brentano goniometer having PSD; Lynx Eye detector. The pattern was recorded at a tube voltage of 40 kV and a tube current of 30 mA, with a step size of 0.02mm and time per step of 1.0 sec over an angular range of 3-4.5. The sample was grounded gently and filled in a sample holder by top loading method. The sample was exposed to the Cuk radiations (1θ= 1.5418 mA) [10]

**FTIR Analysis**

The IR spectrum of drugs in KBr/MeOH is presented in IR spectra's (KBr pellets) of the different polymorphic forms of crystals [11].

**RESULTS**

**Evaluation of cefuroxime axetil and aztreonam crystal**

**Table 1:Evaluation of cefuroxime axetil and aztreonam crystal.**

| | | Cefuroxime axetil | | | | Aztreonam crystal | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Sl. No** | **Evaluation technique** | **Water crystal** | **Methanolic crystal** | **Ethanolic crystal** | **$NH_3$ Diffusion Crystal** | **Water crystal** | **Methanolic crystal** | **Ethanolic crystal** | **$NH_3$ Diffusion Crystal** |
| 1. | Production yield (%) | 12 | 20 | 15 | 45 | 85 | 72 | 82 | 64 |
| 2. | Solubility (mg/ml) | 0.5±0.05 | 0.55±0.05 | 0.65±0.05 | 0.92±0.05 | Insoluble | Insoluble | Insoluble | Insoluble |
| 3. | Melting point | 67±1 | 66±0.5 | 66±1.5 | 66±2 | 227±5 | 225±5 | 224±5 | 226±5 |

**Characterisation of Cefuroxime Axetil by means of FT-IR**

**Figure 1: FT-IR Spectrum of cefuroxime axetil pure drug**

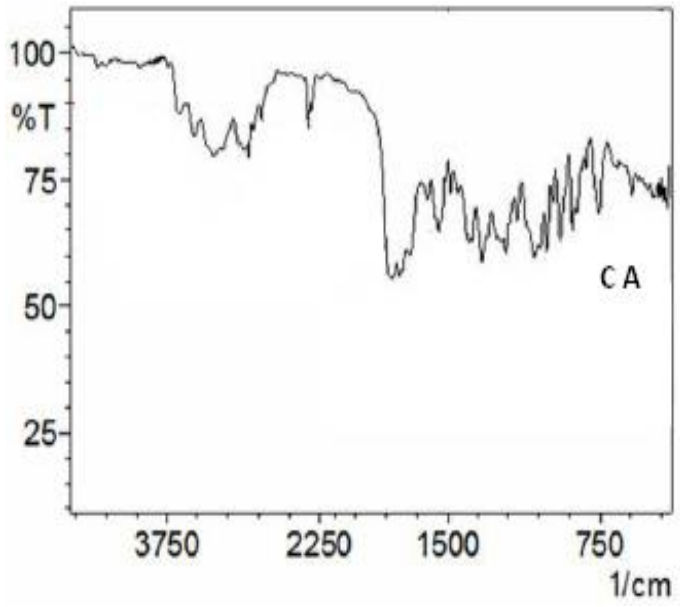
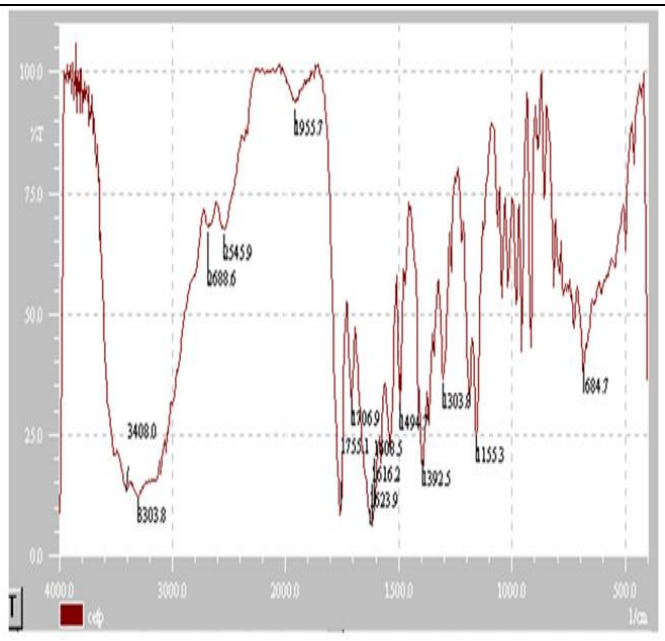



**Figure 2: FT-IR Spectrum of ammonia crystal of cefuroxime axetil**



**Characterisation of Aztreonam by means of FT-IR**

**Figure 3: FT-IR of pure aztreonam**

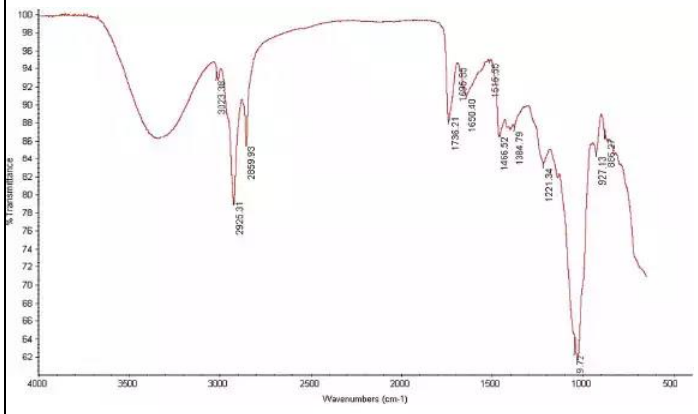
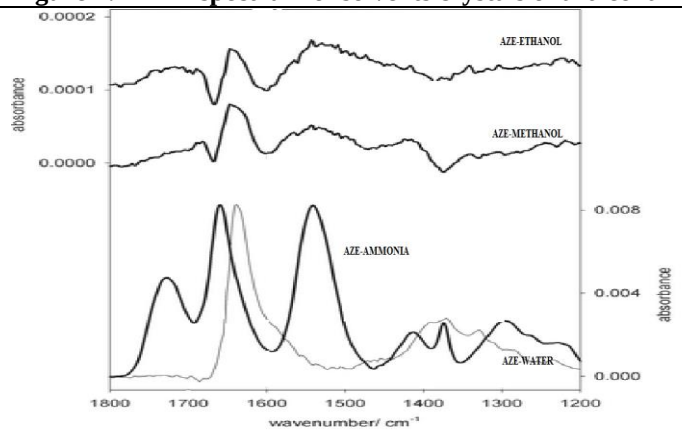
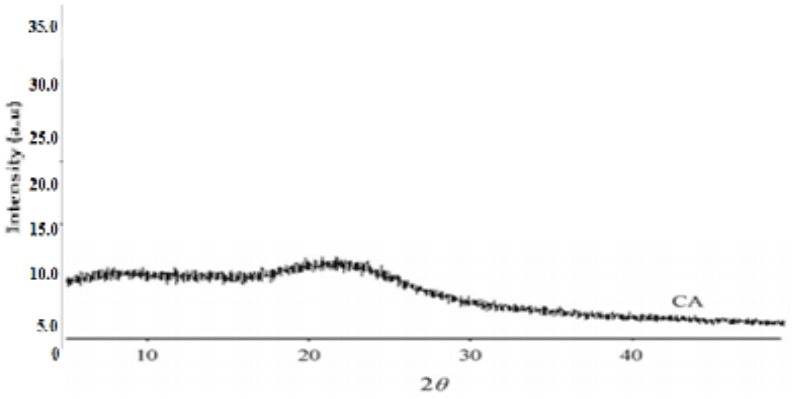



**Figure 4: FT IR spectrum of solvents crystals of aztreonam**



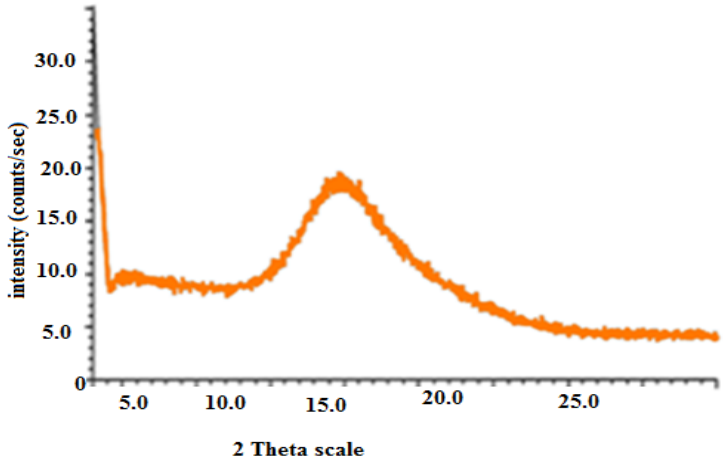

**X-RAY Diffraction Study of Cefuroxime Axetil**

**Figure 5: X-RAY diffraction study of cefuroxime axetil**

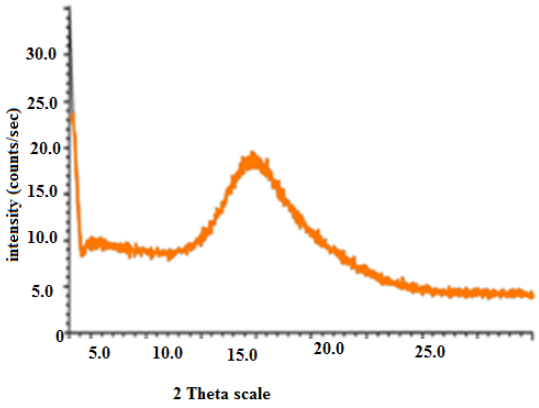



**X-RAY Diffraction Study of Cefuroxime Axetil Ethanol Crystal**

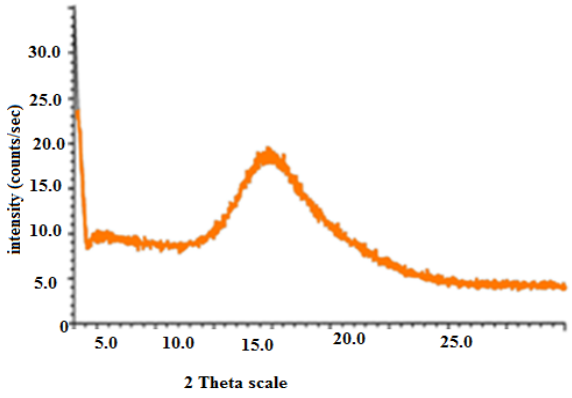
**Figure 6: XRD of Cefuroxime Axetil Ethanol Crystal**



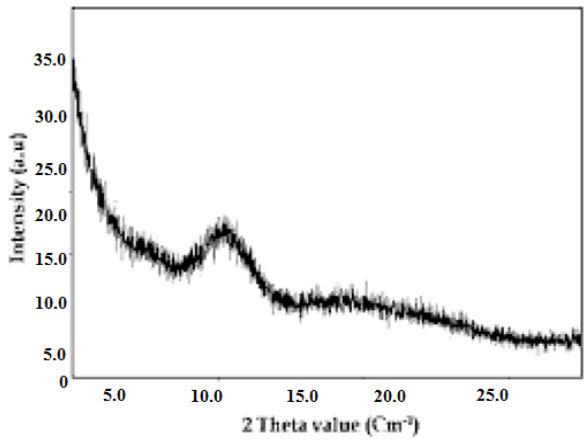
**X-RAY Diffraction Study of Cefuroxime Axetil Methanol Crystal**
**Figure 7: XRD Cefuroxime Axetil Methanol Crystal**



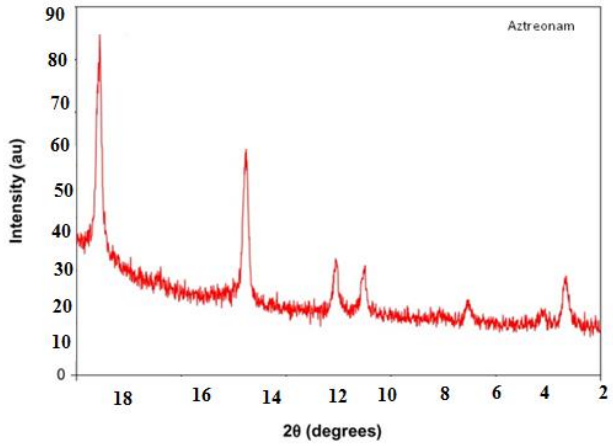

**X-RAY Diffraction Study of Cefuroxime Axetil Water Crystal**
**Figure 8: XRD Cefuroxime Axetil Water Crystal**



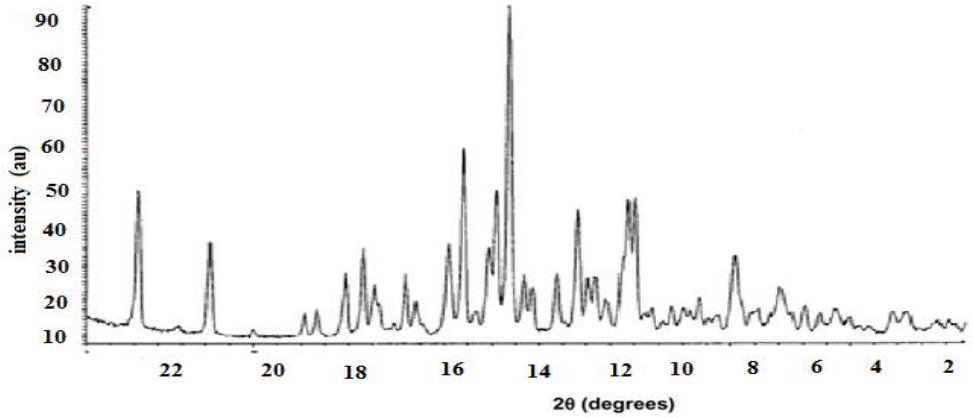

**XRD OF Cefuroxime Axetil –Ammonia Diffusion Crystal**
**Figure9: XRD of Cefuroxime Axetil –Ammonia Diffusion Crystal**




**X-RAY Diffraction Study of Aztreonam**
**Figure 10: XRD of pure aztreonam**

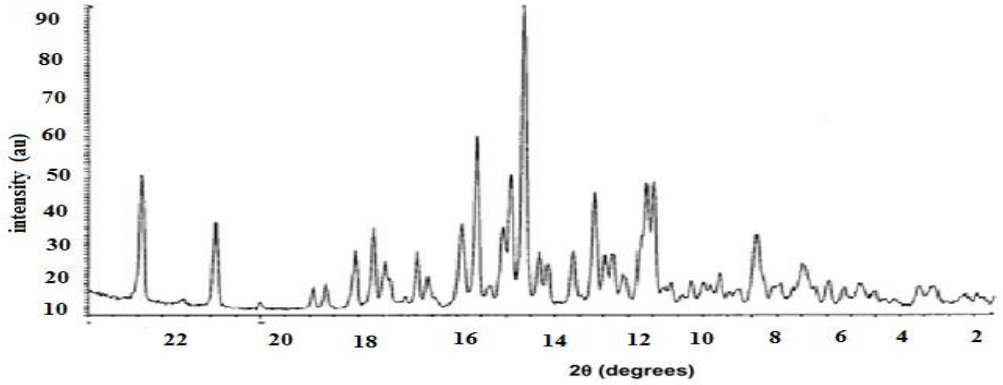



**XRD of aztreonam water crystal**
**Figure 11: XRD of aztreonam water crystal**



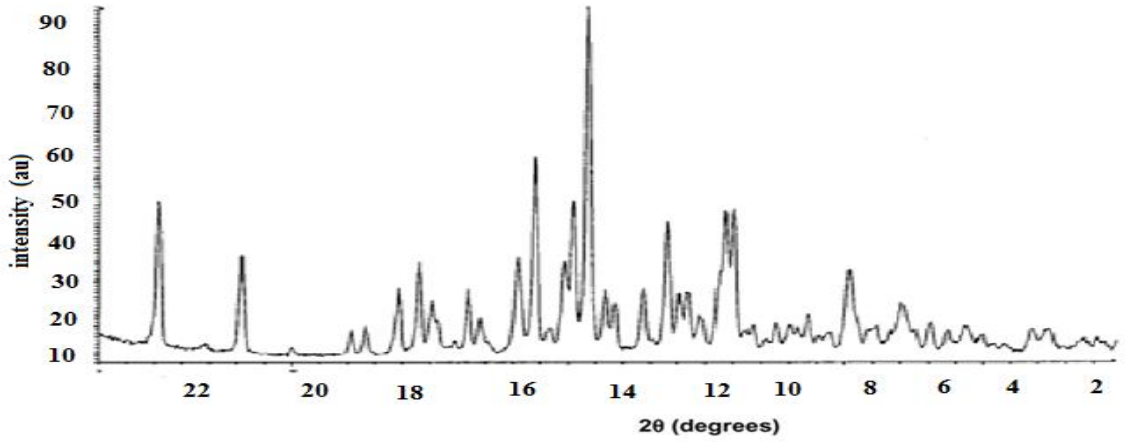
**XRD of aztreonam ethanol crystal**

**Figure 12: XRD of aztreonam ethanol crystal**



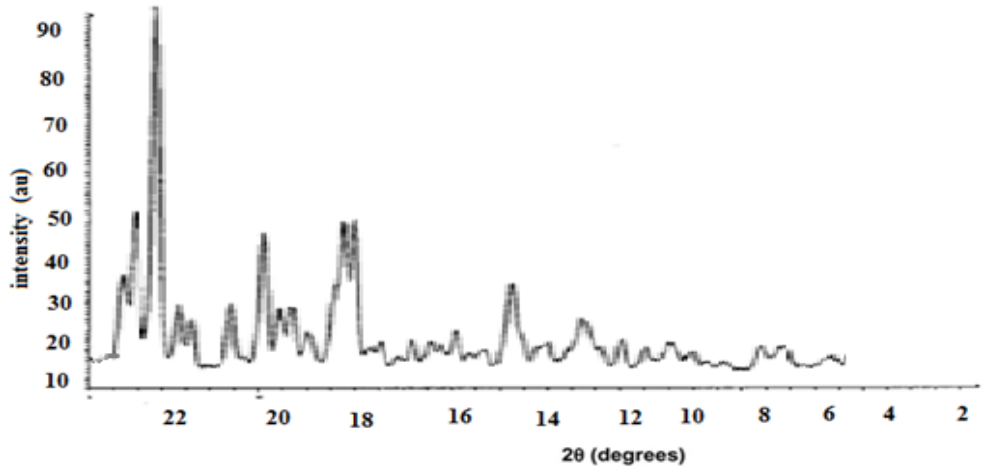

**XRD of aztreonam Methanol crystal**

**Figure 13: XRD of aztreonam methanol crystal**




**XRD of aztreonam ammonia crystal**

**Figure 14: XRD of aztreonam ammonia crystal**



## DISCUSSION

Cefuroxime axetil is amorphous in nature. The drug aztreonam is practically insoluble in water. However, the presence of crystalline phase due to unexcepted crystallization during storage cannot be overruled. Amorphous materials are associated with short coming of physical instability and higher chemical reactivity through they possess maximal solubility.so in the present study four crystalline solids were prepared using different solvent water, ethanol, methanol. Ammonia with acetone and dichloromethane. The crystals prepared were tested for solubility and melting point and the results were given in the table 1. FTIR techniques have been used study the physical and chemical interaction between the drug and solvent used. Figure 1 shown FTIR spectrum of cefuroxime axetil. In Spectrum of cefuroxime axetil, the free asymmetrical and symmetrical N-H stretching modes, thio, azo, cyclic octane, carboxylic acid, hydroxy methyl, amido ester functional groups were present. Figure 2 showed the spectrum of cefuroxime axetil ammonia crystal. The solvent ammonia interaction with CA that show in, N-H stretching at 3408-1616.2cm$^{-1}$. In Figure 3 Spectrum of Aztreonam has been showed. From that spectra, remarking peaks of aztreonam has marked with which are characteristic to the C=O stretching (**1736.21,1696.99,1630.40,1516.55,1455.52,1564.79**), and N-H stretching (**3023.38,3142.71,3061.12,3213.71,3492.15**). The IR spectroscopy technique described herein was developed primarily for analysing polymorphic solvent crystal, but the same procedure can be applied to all solvent. The method involves obtaining the near FTIR of the pure polymorph (ammonia crystal) and then using this spectrum to identify qualitatively and determine quantitatively the polymorphic forms (Aze-ethanol, methanol, water, ammonia) that are present any sample of crystals. The IR spectroscopy has been used only to show different between polymorphic form of crystals. The present work extends the IR technique identification and determination of polymorphic forms of both organic and inorganic crystals.

The XRD pattern of CA showed the absence of significant sharp peaks indicating that the drug is in an amorphous state, which agrees with DSC. This confirmed that the CA was present in the amorphous state in the solid dispersion, and that there was no chemical interaction of CA with the excipients used. The amorphous nature of CA in the prepared dispersion granules ratified the solubility-enhancement potential of excipients used for preparation of dispersions granules.

XRD pattern of cefuroxime axetil ethanol crystal 2θ peak were detected at scattering angles and the interpretations are given in (figure 6).

XRD pattern of cefuroxime axetil methanol crystal 2θ peak were detected at scattering angles and the interpretations are given in (figure 7).

XRD pattern of cefuroxime axetil water crystal 2θ peak were detected at scattering angles and the interpretations are given in (figure 8).

XRD pattern of cefuroxime axetil ammonia crystal 2θ peak were detected at scattering angles and the interpretations are given in (figure 9).

XRD pattern of aztreonam water crystals 2θ peak were detected at scattering angles and the interpretations are given in figure 11. XRD pattern of aztreonam ethanol crystals 2θ peak were detected at scattering angles and the interpretations are given in figure 12. XRD pattern of aztreonam methanol crystals 2θ peak were detected at scattering angles and the interpretations are given in figure 13. XRD pattern of aztreonam ammonia crystals 2θ peak were detected at scattering angles and the interpretations are given in figure 14.

The Powder X-Ray Diffraction Pattern Confirmed Physical Nature of the raw Material and Solvents (Ethanol, Methanol, Water, Ammonia) crystals shown in above figure, x-diffractogram of the raw material of CA didn't show any peak indicating its amorphous nature. Similarly, the above solvent crystals except ammonia others didn't show proper peak indication. But in ammonia crystals of CA have shown less intense peak at **12.09**, **13.52**, **14.89**, **15.21**, **16.72**, **11.52**, **10.23**, **09.81**, (2θ angles) respectively dictating retention of the partial amorphous form of the CA-crystals exhibit well defined characteristic peak manifestation conversion to crystalline forms. The Powder X-Ray Diffraction Pattern Confirmed Physical Nature of the raw Material and Solvents (Ethanol, Methanol, Water, Ammonia) crystals shown in above figure x-diffractogram of the raw material of Aztreonam show more peaks indicating its pure crystalline nature. But in ammonia crystals of aztreonam have shown very high intense peak at **14.76,16.12,23.41,21.78,18.85,15.99**,**11.92**,**08.90** (2θ angles) respectively dictating retention of the pure crystals exhibit well defined characteristic peak.so from above all the four XRD figures ammonia crystals of aztreonam behave very good crystalline nature combined with lysine amino acid. Here the ammonia aztreonam-lysine combination choice for iv infusion formulations.

## CONCLUSION

The pharmaceutics scientist must formulate this material into a dosage form that is homogeneous, callable, stable, and bio available. The single crystal X-ray technique was used to identify the crystal structure of Aztreonam/Cefuroxime Axetil and its polymorphic analogues. These X-ray structures were systematically studied and analyzed for solubility trends with respect to the molecular conformations of different active Aztreonam/Cefuroxime Axetil. The crystals of cefuroxime Axetil and aztreonam were fully identified by powder X-ray diffraction method and characterized by FT-IR spectroscopy techniques. As expected, both solubility and stability are good with ammonia polymorphs but normally these two

properties are inversely related for many polymorphic systems. We have optimized ammonia polymorph for Aztreonam and cefuroxime axetil drugs and other model compounds with greater stability and higher solubility. This comparison provides a direction to crystallize ionic polymorphs of amphoteric drugs for solubility enhancement. In the light of the results of the present study, it is concluded that the crystalline forms of the selected drugs Cefuroxime axetil and aztreonam prepared by ammonia diffusion method did not undergo any phase transition by either formulation variables or manufacturing processes as shown by no changes in their stability and essential physico-chemical properties dependent on crystalline behaviour.

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