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Research Article

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Determination of traces of crystalline cefuroxime axetil content in cefuroxime axetil amorphous drug substance using powder X-Ray Diffraction (PXRD) Technique

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ABSTRACT

Amorphous materials are often preferred in the pharmaceutical industry due to their enhanced dissolution rate and bioavailability. Since the amorphous state is metastable and less thermodynamically stable relative to the crystalline state, there is always the potential for unexpected crystallization during storage. Conversion of amorphous to crystalline state can be evaluated by stability studies. Accurate quantification of crystalline phases present in drug materials has become increasingly imperative, due to stringent regulatory concerns about polymorph characterization. In the present study, a quantification method was developed for the determination of Crystalline phase in Cefuroxime Axetil amorphous phase using X-ray powder diffraction technique. Validation of quantification method was carried out with respect to Specificity, Precision, Linearity, Robustness, Limit of Detection and Limit of Quantification. Validation results were found to be within acceptance criteria.

Key words: *Cefuroxime Axetil, Amorphous phase, crystalline phase, PXRD quantification, Drug substance.*

INTRODUCTION

Amorphous materials are shapeless solids that can be distinguished from crystals by their lack of macroscopic and microscopic properties such as shape, birefringes and fracture mechanism . Amorphous solids have attracted the interest of pharmaceutical scientists because of two major developments (i) a continuous increase in the number of insoluble developmental drug molecules because of the advent of novel methods of synthesis and screening and (ii) the growing attention in regulatory aspects of the pharmaceutical solids. Amorphous form due to absence of an ordered crystal lattice requires minimal energy and thus providing the maximal solubility advantage as compared to the crystalline and hydrated forms of a drug. The 'apparent solubility' and dissolution advantage offered by these systems is a vital approach to enhance bioavailability of poorly water soluble drugs. However, limitations of amorphous systems such as physical instability and higher chemical reactivity, act as an hurdle in their extensive commercialization [1-7].

The use of amorphous materials in any field is associated with some challenges. A vey significant challenge is that the amorphous phase is thermodynamically unstable compared to any crystalline phase of the same material. During manufacturing operations and/or storage amorphous forms are likely to revert into the stable or a metastable crystalline form if they are not adequately stabilized. X-ray powder diffraction known as XRPD is a powerful tool

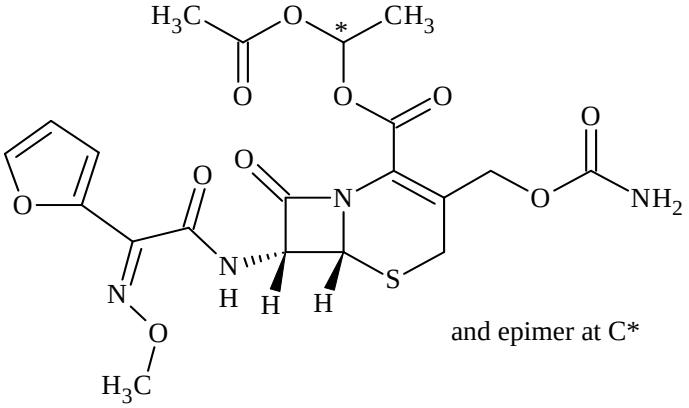
in pharmaceutical industries for phase quantification [8-9] Advantages of XRPD method are simplicity, measurement at room temperature and the non-destructive nature [10].

The scope of the present study was to demonstrate the application of PXRD in the detection of low level crystalline content in Cefuroxime axetil amorphous samples as per the regulatory requirements [11]. International Conference on Harmonization (ICH) Q6A guidelines provides guidance on, when and how polymorphic forms should be monitored and controlled [12].

MATERIALS AND METHODS

Cefuroxime axetil (1-Acetoxyethyl (6*R*,7*R*)-3-[(carbamoyloxy)methyl]-7-{[(2*Z*)-2-(2-furyl)-2-(methoxy imino) acetyl]amino}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate) is a second generation oral cephalosporin antibiotic discovered by Glaxo. It is an acetoxyethyl ester prodrug of cefuroxime which is effective orally. Its molecular formula is $C_{20}H_{22}N_4O_{10}S$, and it has a molecular weight of 510.48 [13,14].

Cefuroxime axetil is in the amorphous form and has the chemical structural formula as in Figure-1



Molecular Formula : $C_{20}H_{22}N_4O_{10}S$
Molecular Weight : 510.47

Figure – 1

Cefuroxime Axetil amorphous and Crystalline samples manufactured at Orchid Chemicals and Pharmaceuticals Ltd, Plant at Alathur, Chennai, India used in the study.

POWDER X-RAY DIFFRACTION

The X-ray powder diffraction pattern was reported on Bruker AXS D8 ADVANCE, equipped with Bragg-Brentano goniometer having LynxEye detector. The pattern was recorded at a tube voltage of 40 kV and a tube current of 30 mA, with a step size of 0.02° and time per step of 1.0 sec over an angular range of 3- 45° 2θ. The sample was grounded gently and filled in a **PMMA** sample holder by top loading method. The sample was exposed to the **$CuK_{\alpha 1}$** radiations (λ = 1.5406 °A).

SAMPLE PREPARATION

Method discussed in this report was developed for the determination stable crystalline form in amorphous Active Pharmaceutical Ingredients (API). The Cefuroxime axetil crystalline sample was assumed to represent a 100% crystalline phase upon positive comparison to the simulated powder patterns. The amorphous material was assumed to be 100% amorphous if no crystalline peaks were observed. Amorphous material was stored at 4°C. To determine the limit of detection in each case, blends using the amorphous material and its related known crystalline phase, were prepared at different concentration levels by using sample blender. All standard mixers are expressed as % crystalline phase in Amorphous form.

METHOD DEVELOPMENT

The purpose of the XRPD method is to demonstrate that the test samples contain Cefuroxime axetil amorphous material without evidence of any crystalline materials, within the limits of detection. A “low” limit of detection is desired, with the eventual desire to relate degree of crystallinity to product performance, such as dissolution rate.

The method, including interpretation of the powder patterns, must be able to be run and analyzed reproducibly by several scientists, and possibly may need to be transferred to a manufacturing site. The test samples are defined as API that is amorphous by X-ray. There is a possibility that some of the samples will also contain amorphous phase that has transformed to a crystalline phase. Thus, the expected PXRD pattern will be an amorphous halo, sometimes with peaks due to crystalline phase. The limit of detection was determined by spiking the standard crystalline phase at lower levels in the amorphous phase.

The characteristic peak of cefuroxime axetil crystalline form at 7.7° 2theta was chosen to check its absence in the amorphous sample. By using the standard mixtures of amorphous and crystalline Cefuroxime axetil and varying the several instrumental parameters a précised and accurate PXRD method was developed. The following instrumental parameters are finalised for this method.

The Bruker D8 Advance with Lynxeye detector and k alpha radiation (1.5406A°) configuration included power of 40 kV x 35 mA, 1° divergence lit, 3.0 mm anti-scattering slit, 0.0003° step size, 1sec time/step, Nickel filter with 60 rpm sample rotation speed.

RESULTS AND DISCUSSION

Pure crystalline and amorphous forms were characterized by PXRD as shown in **Figure-2** . In PXRD Pure crystalline form was characterized with high intensity characteristic peaks (7.7 ° 2theta) with a stable baseline.

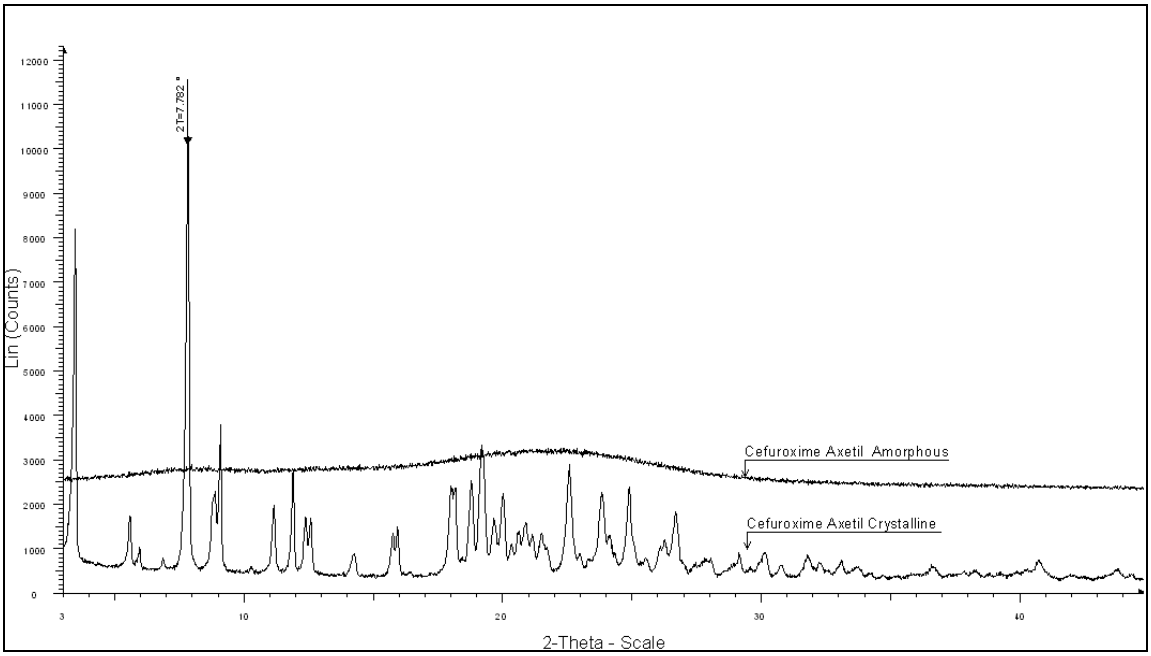


Figure – 2

The standard mixtures of crystalline Cefuroxime axetil in amorphous form at the various level of concentration, 0.1, 0.25, 0.5, 1.0, 2.0, 3.0 and 5.0% , were prepared and analyzed in PXRD in the range of 6.8 to 8.6° 2theta for the detection of crystalline content. The diffractogram of crystalline content in amorphous cefuroxime axetil drug substance at different levels are shown in **Figure 3.** The linearity chart between the concentration and the area of the crystalline form in Amorphous is shown in **Figure- 4** and **Table 1.**

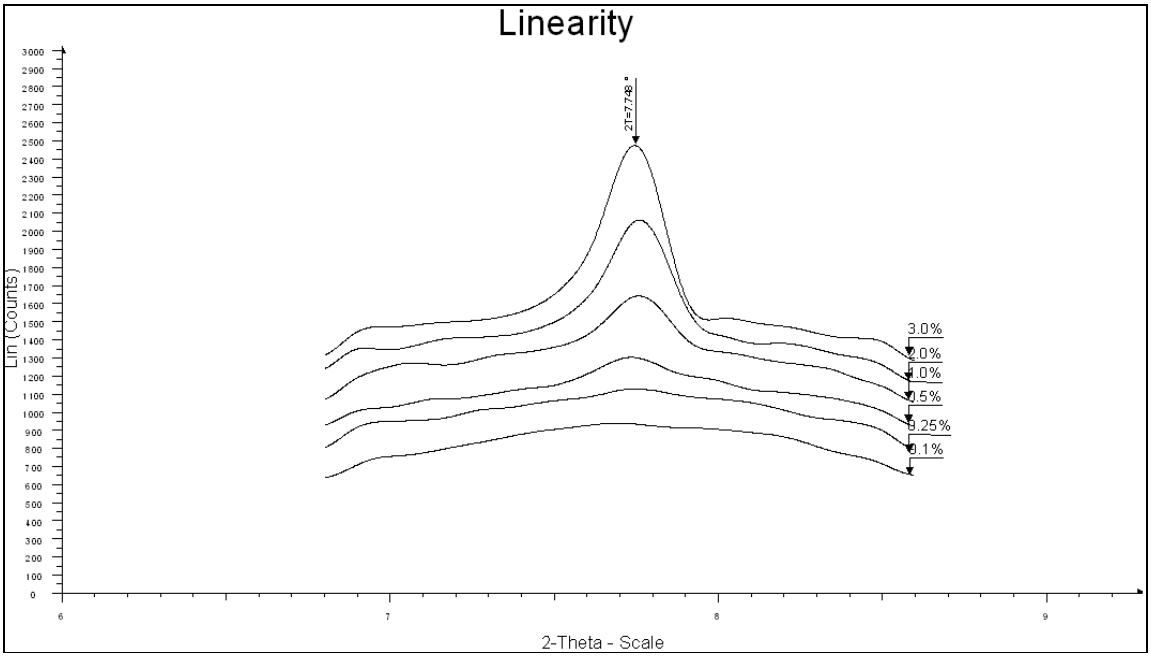


Figure-3

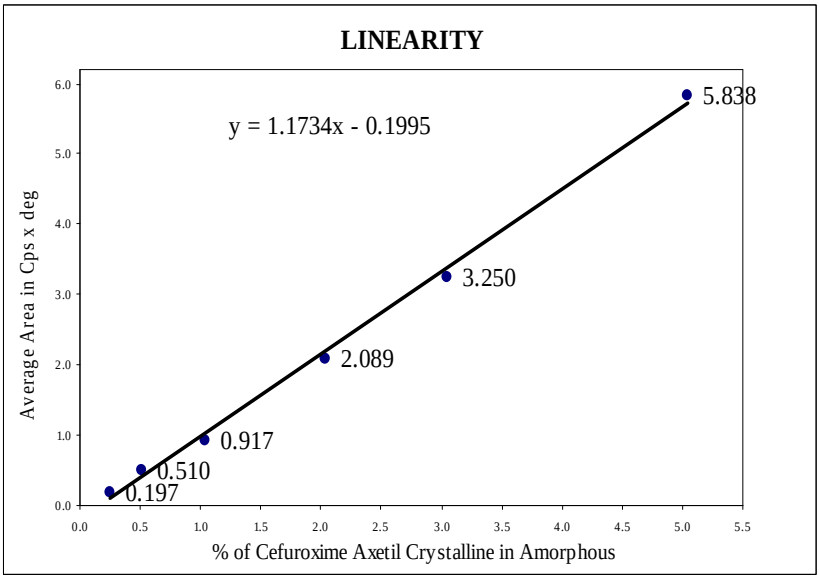


Figure - 4

Table – 1

Cefuroxime Axetil Crystalline form spiked in Cefuroxime Axetil Amorphous (%w/w)	Area in Cps x Deg at about 7.7° (2θ)
0.25	0.197
0.52	0.510
1.04	0.917
2.04	2.089
3.05	3.250
5.04	5.838
Slope	1.1734
STEYX	0.116
Intercept	-0.1995
Correlation Coefficient	**0.9984**

The regression line shows the linear relationship between the concentration and the area of the crystalline form. The crystalline content in any unknown sample can be detectable and quantifiable by using this calibration curve.

PREDICTION OF LOD

Several methods were evaluated to obtain a reproducible analysis technique to observe the limit of detection (LOD) of a crystalline Form in Amorphous material [15]. With the noise generated by the diffuse disorder scattering from the amorphous material, reproducible interpretation of a peak is difficult, but necessary for a validated method. The FDA published guidelines [16] for LOD methods, which suggested LOD can be determined by visual evaluation, signal-to-noise evaluation and Standard Deviation of the Response and Slope. This last method is not possible for this case because the signal is not linear with the degree of crystallinity. Often, a sufficiently trained scientist can recognize a peak, which would make the visual evaluation possible. However, validation of that visual recognition is close to impossible. Therefore, a numeric approach to analysis must be taken. Smoothing algorithms, background subtraction and peak finding routines were evaluated. The Fast Fourier Transform (fft) smoothing algorithms work well with crystalline materials but tend to introduce low intensity peaks for an amorphous material, which lead to problems distinguishing real peaks from fft-generated peaks. The traditional smoothing methods, including the Sovitsky-Golay least squares smoothing algorithm, tended to reduce the detectability of low intensity peaks. Background subtraction techniques often did not adequately describe the background of a halo. The limit of detection of the known most stable crystalline form for each system was determined by making mixtures of crystalline Form in the Amorphous Material at lower levels . The limit of detection was determined to be 0.25% by observing that 0.25% crystalline form was repeatedly detected, but that lower levels were not reproducibly detected.

LOD PRECISION

This repeatability is confirmed by calculating the % RSD for the area counts of crystalline form for consecutive analysis at LOD level as in Table - 2. The diffractograms of LOD level Precision is shown in Figure - 5.
(Acceptance Criterion: the % RSD should be not more than 33).

Table - *2*

LOD (%w/w) - 0.25%	
S.No	Area in Cps x Deg at about 7.7° (2θ)
1	0.271
2	0.277
3	0.304
4	0.19
5	0.181
6	0.192
Average	0.2358
SD	0.054
%RSD	**22.9**

PREDICTION OF LOQ

The Limit of Quantization (LOQ) of Cefuroxime Axetil Crystalline form spiked in Cefuroxime Axetil Amorphous drug substance is evaluated by spiking Cefuroxime Axetil Crystalline form in Cefuroxime Axetil Amorphous drug substance at double the concentration of LOD value (0.25%). The area counts per second x degrees were obtained from the diffractogram at about 7.7° (2θ) and the precision was calculated. The result is shown in **Table 3** and a representative diffractogram at the LOQ concentration is shown in **Figure. 6.**
(Acceptance Criterion: the % RSD should be not more than 10).

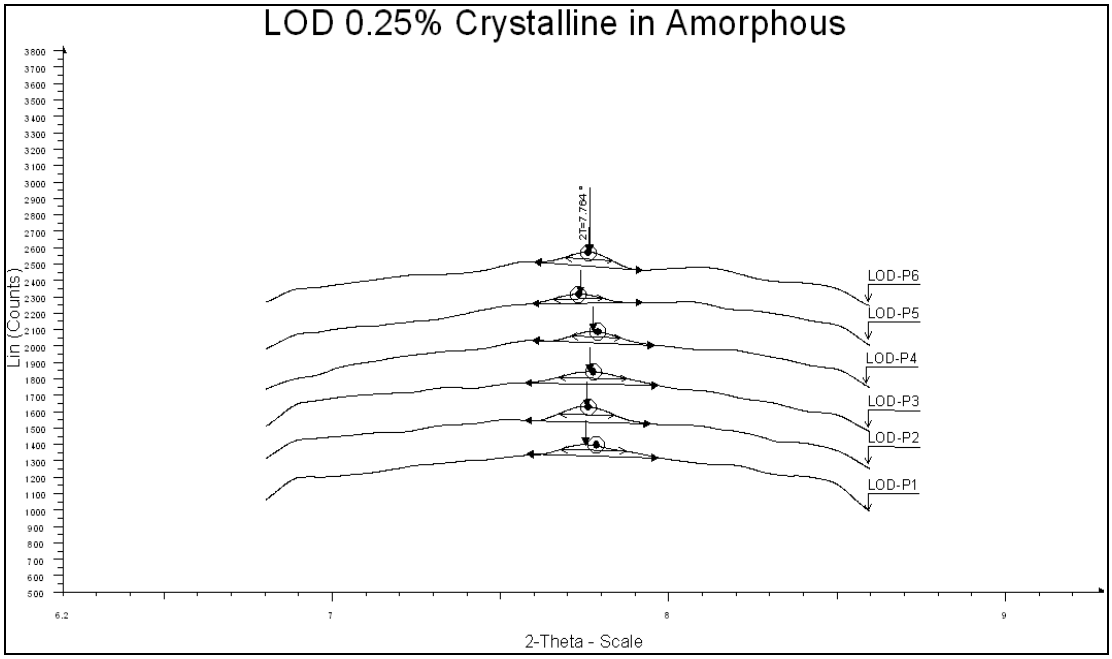


Figure-5

Table – 3

LOQ (%w/w) - 0.5%	
S.No.	Area in Cps x Deg at about 7.7° (2θ)
1	0.531
2	0.555
3	0.59
4	0.511
5	0.484
6	0.51
Average	0.5302
SD	0.0377
%RSD	**7.11**

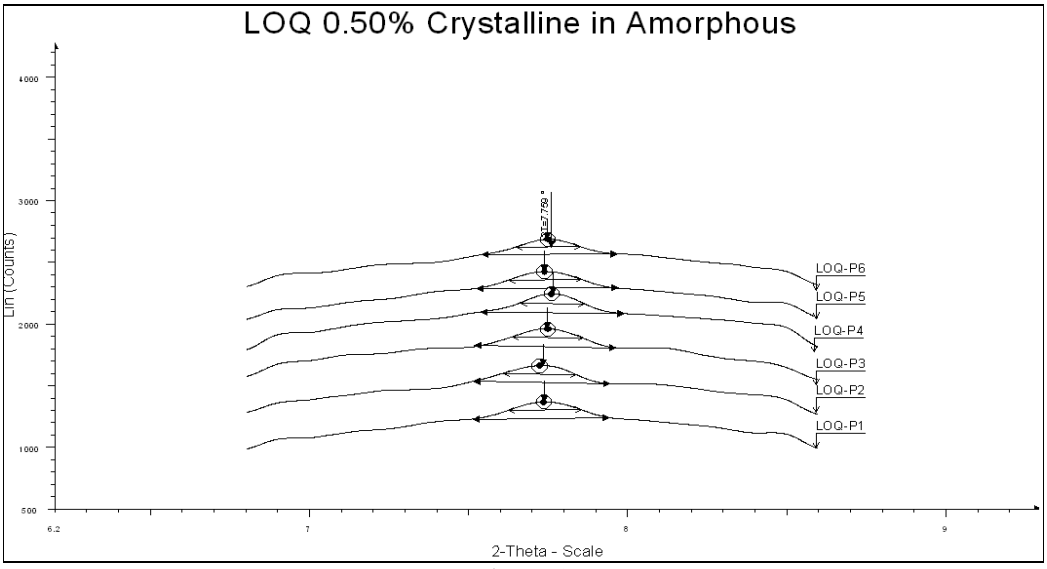


Figure – 6

RUGGEDNESS OF THE METHOD

The ruggedness of the proposed method was determined by spiking Cefuroxime Axetil Crystalline form in Cefuroxime Axetil Amorphous form drug substance at about the given LOQ concentration level six times each by two different analysts on different days as per the proposed method. The overall Average, Standard Deviation and % RSD of the data shown in Table- 4 indicates that the method is rugged

(Acceptance Criterion: overall % RSD should be not more than 10)

Table – 4

S.No.	LOQ Level	
	0.50%	
	Area in Cps x Deg at about 7.7° (2θ)	
	First Analyst	**Second Analyst**
1	0.531	0.514
2	0.555	0.515
3	0.59	0.517
4	0.511	0.524
5	0.484	0.487
6	0.51	0.576
Average	0.5302	0.5222
SD	0.0377	0.0293
% RSD	0.711	5.61
Overall Average	0.526	
Overall SD	0.0324	
Overall % RSD	**6.16**	

CONCLUSION

The XRPD method described in this study was developed to determine trace levels of Crystalline phase in Cefuroxime axetil amorphous phase as per regulatory requirements. Pure crystalline and amorphous standards were prepared and used for the determination of limit of detection. Validation of quantification method was carried out with respect to Specificity, Precision, Linearity, Robustness, Limit of Detection and Limit of Quantification and this method can be used in manufacturing site to check the crystalline contamination in amorphous Cefuroxime axetil.

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