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Determination of residual solvents and investigation of their effect on ampicillin trihydrate crystal structure

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Abstract

In the present work, the relationship between residual solvents concentration and ampicillin trihydrate crystals stability has been investigated. The amounts of residual solvents determined by GC, X-ray powder diffraction (XRPD) and Fourier transform infrared spectroscopy (FT-IR) were used for characterization of solid state. The obtained results have shown good relationship between concentration of methylene chloride (as a critical residue solvent) and degree of ampicillin trihydrate crystallinity. As with the increasing methylene chloride concentration in the sample the degree of crystallinity decreased after stability test. From this relationship, critical concentration of methylene chloride into the ampicillin trihydrate is obtained and the results can be used for improving the large-scale production of ampicillin trihydrate.

Keywords: Ampicillin trihydrate; GC; Residual solvent; Crystal stability; XRD

1. Introduction

The presence of solvents is essential in all steps of pharmaceutical process (reaction, separation and formulation). A typical drug synthesis route consists of three to eight reaction steps and four or more different solvents may be employed in the process [1]. The main function of the solvent in reaction steps is solubilization. However, the selectivity, rate and yield of the synthetic reactions can be significantly affected by the type and amount of the solvent [2,3]. Extraction is one of the common methods of separating the products of synthetic reactions from reagents. In the formulation, the presence of a solvent may affect the kinetics of crystallization and the shape of the products crystals (morphology). Crystallization from solvents is commonly used for the purification of drugs during their final stages of manufacturing and crystal morphology is an important factor that determines the products' quality (e.g. dissolution rate and stability) [4–6].

The solvents are not completely removed by practical manufacturing techniques and their traces may remain in the final products. For just toxicological reasons, drug manufacturers are increasingly required to monitor and limit the presence of residual solvents in their products [7]. Certain types of solvents of known toxicity and environmental hazard (e.g. benzene and chlorocarbons) are not permitted to be used in the manufacture of pharmaceuticals. At the same time, the maximum content of individual solvents in the drugs is regulated, since the number of acceptable solvents is very limited [7,8]. The presence of these unwanted chemicals even in small amounts may influence the efficacy, safety and stability of the pharmaceutical products [9]. Pharmaceutical products often exist in several polymorphic forms with

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narrow stability region [1]. The size of crystals sometimes determines the quality, especially the stability of bulk drugs. Large-size crystals can entrap a minute amount of chemicals (solvents) from the mother liquor in the crystallization step, which ultimately causes the degradation of the drug [9].

The different pharmacopoeias, such as the British pharmacopoeia (BP) and the United States pharmacopoeia (USP) have recently noticed the residual solvent toxicological properties and reported different analysis methods for them [10]. But, they have not mentioned anything about the stability of pharmaceutical products in the presence of residual solvent. Also, many authors have published many articles about the analysis of residual solvent in the pharmaceutical products [11–17], the polymorphism of pharmaceuticals [18–23] and the influence of solvent on the pharmaceutical morphology [24]. A few papers about influence of the residual solvent on the stability of the pharmaceutical crystals have been reported. For example, the influences of triethyl amine (TEA) as a residual solvent and cadmium ($\prod$) ions on the stability and degradation of ampicillin trihydrate have been reported [25,26]. In other reports, the kinetics of ampicillin degradation and thermal decomposition of penicillins have been investigated [27,28].

Ampicillin ($C_{16}H_{19}N_3O_4S$) is a common antibiotic that is effective against a wide variety of Gram-positive and Gram-negative organisms [24]. Various hydrated forms of ampicillin have been reported including a monohydrate, dihydrate and trihydrate [29]. However, it is probable that the trihydrate is a stable hydrate of defined composition versus the other forms.

Recently, many experimental techniques, such as DSC, XRD, Fourier transform infrared spectroscopy (FT-IR) and TGA have been used for studying the pharmaceutical solid state [29–33]. In this report, the influence of methylene chloride as a residual solvent on stability of ampicillin trihydrate crystals has been studied. GC and HPLC were used as powerful instruments for analysis of residual solvent in the ampicillin and determination of ampicillin samples potency, respectively. XRD and FT-IR were used to characterize the samples solid state and the accelerated heat stability test was used for stability test method.

2. Experimental

2.1. Materials

Ampicillin trihydrate standards were obtained from Sigma (St. Louis, MO, USA). Methylene chloride (MC), 2-propanol (IPA), methyl isobutyl ketone (MIBK), triethylamine (TEA) and 1-propanol used as internal standard, ammoniac (NH_3), HPLC-grade methanol and hydrochloric acid were purchased from Merck (Darmstadt, Germany). Ampicillin trihydrate real samples were obtained from Zakaria Pharmaceutical Company (Tabriz, Iran).

2.2. Powder X-ray diffraction (PXRD)

The powder X-ray diffraction patterns of the ampicillin trihydrate solid phase were determined using a X-ray generator (PW 1130/00) and goniameter (PW1050, Philips, Almelo, the Netherlands) with Cu Kα radiation (wavelength: 1.541 Å) at 30 mA and 40 kV with 2θ increasing in the rate of $3^\circ\ min^{-1}$. Counts were accumulated for 1 s at each step. Each sample was packed in an aluminum holder and the instrument was operated between an initial and final 2θ angle of 4 and 40°, respectively, in increment of 0.052θ.

2.3. Karl–Fischer titrimetry (KFT)

The relative amounts of water, expressed as % (w/w), and as the number of moles of water per mole of anhydrous ampicillin, in the ampicillin powders were determined using Mitsubishi moisture meter (Model CA-05, Mitsubishi Chemical Industries Limited, Tokyo, Japan). The sample (6–10 mg) was accurately weighed and quickly transferred to the titration vessel to measure the water content.

2.4. Fourier transform infrared spectroscopy (FT-IR)

FT-IR was carried out by an IFS 66/S spectrometer from Bruker (Karlsruhe, Germany). FT-IR spectra of sample were obtained using KBr pellet prepared with a press (under a hydraulic pressure of 10 t for 30 s) after careful grinding of each sample with KBr. Spectral width was 500–4000 cm^{-1} and spectral resolution was 4 cm^{-1}.

2.5. Sample preparation

2.5.1. Recrystallized samples

A 5 g of pure ampicillin trihydrate powder (with very low and known residual solvent) was weighed, transferred to an Erlenmeyer flask and dissolved in 25 mL of water (pH = 2) by soaking and agitating with magnetic stirrer at ambient temperature. This solution was suspended in an ultrasonic bath followed by addition of 0, 1, 2, 4, etc. milliliters of methylene chloride (MC) for preparation of samples (Ampi1–Ampi7) of different concentrations. The recrystallization of ampicillin trihydrate was accomplished at pH around 4–5 by gently adding NH_3 to the suspension solution. The resulting precipitation was filtered by sinter glass, and then ampicillin trihydrate crystals were washed with 50 mL of water/isopropyl alcohol (15:85) and were dried at room temperature. Then these samples were used for stability test and sample preparation for GC analysis.

2.5.2. Powder samples

These samples (Ampi8–Ampi12) were prepared by adding methylene chloride to sample powder in different concentrations (500–3000 ppm) and sealed with rubber and capped. Then these samples were used for GC analysis and solid-state characterization.

Table 1
GC and Karl–Fischer results for ampicillin trihydrate samples

S.P.T.[a]	Sample name	Residual solvents			
		MC	IPA	TEA	K_f (%)
R.S.[b]	Ampi-1	ND	430	ND	13.73
	Ampi-2	1050	600	ND	13.47
	Ampi-3	1200	370	ND	13.66
	Ampi-4	1350	280	ND	13.71
	Ampi-5	1500	350	ND	13.59
	Ampi-6	1800	500	ND	13.50
	Ampi-7	2100	450	ND	13.81
P.S.[c]	Ampi-8	500	ND	ND	13.47
	Ampi-9	1000	ND	ND	13.47
	Ampi-10	1500	ND	ND	13.47
	Ampi-11	2000	ND	ND	13.47
	Ampi-12	3000	ND	ND	13.47

[a] S.P.T.: sample preparation type.
[b] R.S.: recrystallized samples.
[c] P.S.: powdered samples.

2.5.3. Sample preparation for GC analysis

A 3 ml volume of NaOH (2N) was added to 1 g of ampicillin trihydrate samples (obtained in pervious sample preparation step) in a 10 mL glass tube to dissolve the samples completely and 3 mg 1-propanol was added as an internal standard. The glass tube was capped and kept at 4 °C before GC analysis to avoid methylene chloride evaporation.

2.6. Chromatographic conditions

Analysis of the residual solvents was carried out on a Varian Model 3600 gas chromatograph (Varian Iberica, Madrid, Spain) equipped with a split/splitless capillary injection port and flame ionization detector (FID). Separations were performed on a CP-Sil 5 Capillary column (25 m × 0.32 mm (i.d.) × 0.52 μm film thickness). The following conditions were used: injector temperature, 110 °C; detector temperature, 250 °C; oven temperature, 50 °C, then increased by the rate of 10 °C min^{-1} to 160 °C, and this temperature remains for 2 min; carrier gas, helium; at a flow rate of 1 mL min^{-1}; injector volume, 1 μL and injection were made in split mode (split ratio, 1:50). A Star Chromatography Workstation version 4.0 was used for acquiring and processing the data.

The HPLC system consisted of a 616E pump, a 996 photodiode array (PDA) detector and a degasser module; data were acquired and processed using a Millennium software version 2.1 (all from Waters, Milford, MA, USA). The chromatographic separations were carried out on Spherisorb (Waters, Milford, MA, USA) C-18 columns (250 mm × 4.6 mm (i.d.), with a particle size of 5 μm). The analyses were performed using a mobile phase composed of water/methanol (60:40). The injection volume was 20 μL, and mobile phase flow rate was 1 mL min^{-1}. The detections were carried out at 254 nm.

2.7. Stability method

Accelerated heat stability test was chosen for this study and a 1 g sample was weighed, transferred to a glass vial (10 mL) and sealed with high-quality rubber. Then this vial was kept at constant temperature (80 °C) in an oven for 48 h [25]. Also, for investigation of the time effect in stability test method, the samples were kept at 80 °C in an oven for 4, 20, 48 and 65 h. Then the samples were quickly removed and allowed to cool to ambient temperature in a desiccator. The cooled samples were used for all steps of experiments.

3. Results and discussion

3.1. Solvent residue analysis

The manufacturing process for ampicillin trihydrate requires more solvents (such as methylene chloride as a reaction solvent). Thus, there is always a chance of having trace of these solvents in the final products. In crystallization or recrystallization step, crystals can entrap a minute amount of these solvents from the mother liquor and these solvents are not completely removed by practical manufacturing techniques and their traces may still remain in the final products. All the obtained samples in the sample preparation step were

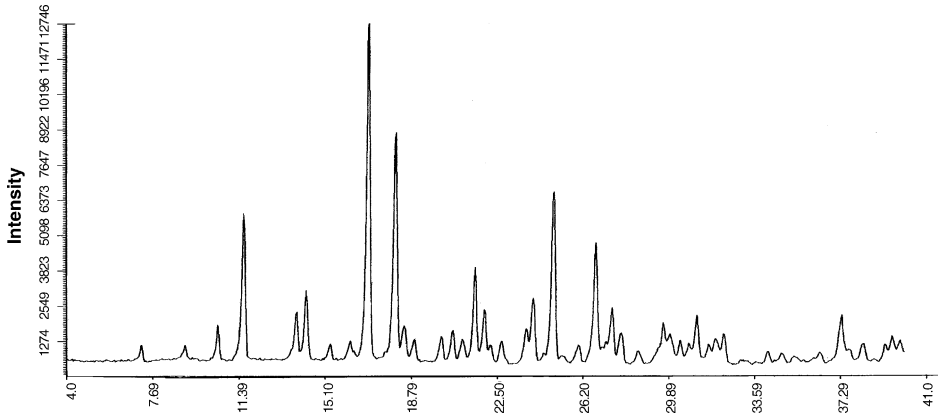

Fig. 1. XRD pattern of ampicillin trihydrate before stability test.

Table 2
Concentration of methylene chloride into the ampicillin trihydrate samples, their color change and XRD peak intensity after stability test

Sample name	Methylene chloride (ppm)	Color	Peak intensity
Ampi1	ND	White	12752
Ampi2	1050	White	13257
Ampi3	1200	White	12672
Ampi4	1350	White	11784
Ampi5	1500	Light yellow	6578
Ampi6	1800	Yellow	3458
Ampi7	2100	Dark yellow	1467
Ampi8	500	White	13547
Ampi9	1000	White	12478
Ampi10	1500	White	14658
Ampi11	2000	White	13584
Ampi12	3000	White	12987

analyzed by GC. The GC and Karl–Fischer results are given in Table 1. Results show that all of the samples have equal content of water, low level of other residual solvents but different content of methylene chloride.

3.2. Accelerated heat stability test

When ampicillin trihydrate was subjected to open vial at ambient pressure, at 80 °C for 48 h, there was a change in the X-ray diffraction pattern. Shefter et al. (1973) dehydrated ampicillin trihydrate isothermal at 83 °C and observed that the dehydrated phase was X-ray amorphous [31]. When ampicillin trihydrate was subjected to ∼140 °C in a sealed vial, it did not reveal any pronounced changes in XRD pattern [32]. For these reasons, this temperature (80 °C) was chosen for accelerated heat stability test in a sealed vial.

3.3. XRD characterization

All the prepared samples were characterized by powder X-ray diffractometery before and after stability test. The obtained results showed no changes in the solid state of ampicillin trihydrate before stability test and their XRD patterns matched that of ampicillin trihydrate reported in the literature. A typical XRD pattern of ampicillin trihydrate is present in Fig. 1.

When samples were kept at 80 °C for 48 h, some of them showed color change from white to yellow. These samples have high contents of methylene chloride and equal contents of water and other residual solvents. Thus, water and other residual solvents do not play critical solvent role. Table 2 shows the concentration of methylene chloride in the

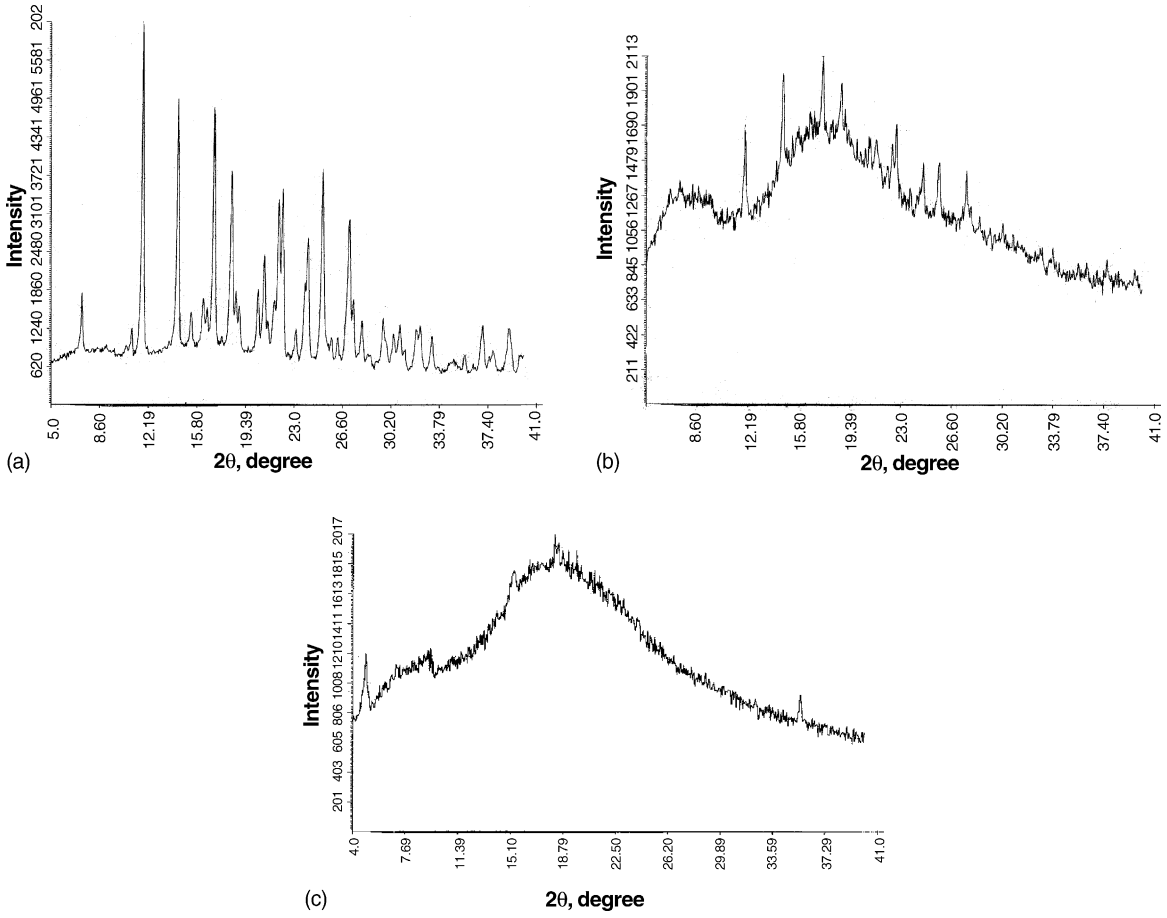

Fig. 2. XRD pattern of: (a) Ampi4, (b) Ampi6 and (c) Ampi7, after stability test.

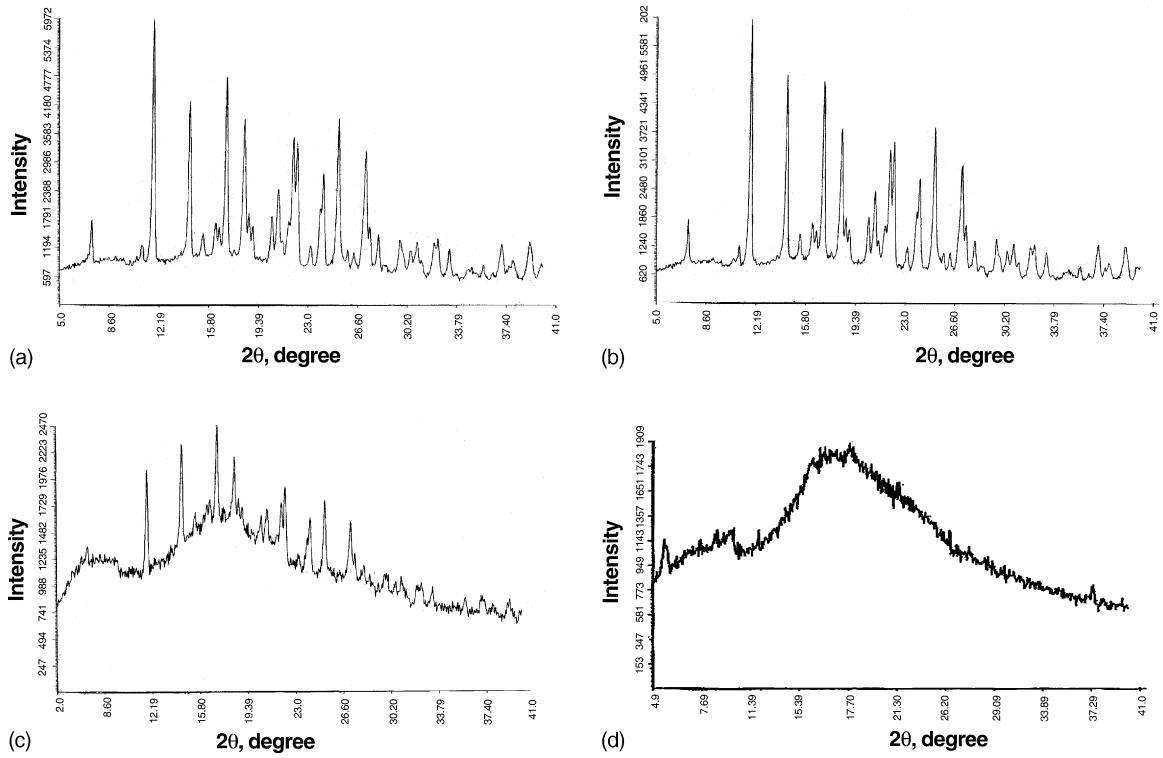

Fig. 3. XRD pattern of Ampi6: (a) 4, (b) 20, (c) 48 and (d) 65 h, after stability test.

samples, their color change after stability test and the peak intensity in their XRD patterns.

These results show that when the concentration of methylene chloride into the samples is more than 1500 ppm, the color change occurs and these samples are unstable. XRD results show that the increase in methylene chloride concentration causes the decrease in degree of crystallinity of the sample (peak intensity in Table 2) in the XRD pattern of samples. When the methylene chloride concentration is more than 2000 ppm in the sample, the degradation of sample is completed. Fig. 2 shows the XRD pattern of these samples.

What is the mechanism of degradation in the presence of methylene chloride? To answer this question, two different types of samples were prepared. In the first samples (Ampi1–Ampi7), the methylene chloride was added in the recrystallization step and entrapped into the ampicillin crystal structures. In the second samples (Ampi8–Ampi12), methylene chloride was added to ampicillin powder at different levels. XDR results show that all of the second samples are stable and have the same XRD patterns before and after stability test. These results show that when ampicillin crystals entrap methylene chloride they become metastable, especially under high-temperature conditions. Under high-temperature condition, the methylene chloride molecules can be removed from ampicillin trihydrate and crystals defect occurred. This phenomenon can be attributed to major degradation factor.

Time effect in stability test method was investigated for one of the samples (Ampi6) and the degradation process was followed by XDR in various times of stability test. The obtained results in Fig. 3 show that the degree of sample crystallinity decreases with increase in stability test time.

3.4. HPLC analysis

The stability of samples was followed by HPLC and determined the assay of all samples before and after stability test. The HPLC results are presented in Table 3. These results show that the degradation occurs when the concentration of methylene chloride is more than 1500 ppm in the ampicillin trihydrate crystals (and recrystalized samples) and these results were in agreement with the XRD observations. Also, these results show that when the loss of potency is more than 30%, the color change occurs.

Table 3
HPLC assay results for some ampicillin trihydrate samples after and before stability test

Sample name	HPLC assay (%)	
	Before stability test	After stability test
Ampi4	88.45	86.25
Ampi5	87.20	51.24
Ampi6	86.50	21.78
Ampi7	86.20	13.25

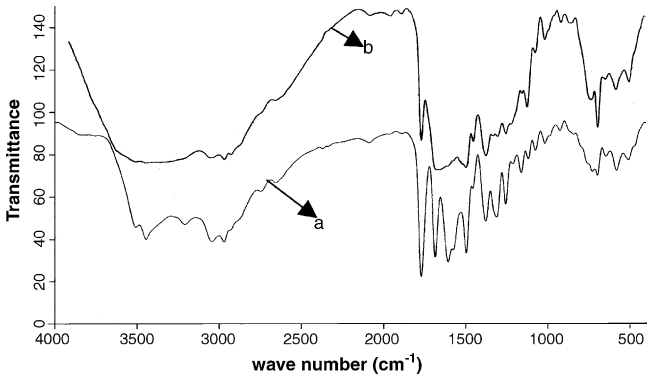

Fig. 4. FT-IR spectrum of Ampi6: (a) before and (b) after stability test.

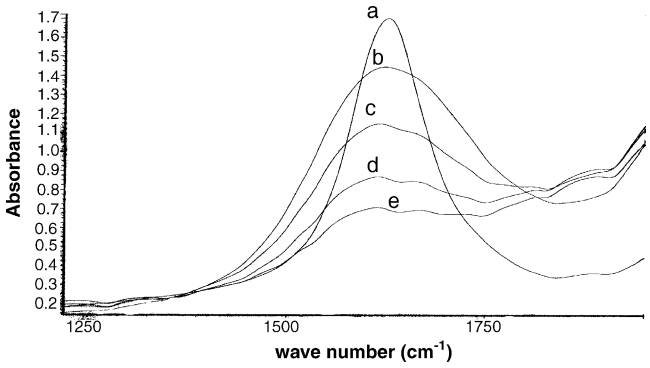

Fig. 5. Comparison of C=O band intensity in different times of stability test for Ampi6: (a) 0, (b) 4, (c) 20, (d) 48 and (e) 65 h.

3.5. FT-IR analysis

Typically the FT-IR spectrums of Ampi6 before and after stability test are shown in Fig. 4. It is evident from comparison of the two spectra that each of this exhibits a characteristic spectrum in the IR fingerprint region (1800–600 cm^{-1}). The obtained spectrum after stability test in Fig. 4 shows that β-lactam ring plays a critical role in samples degradation and its C=O band is a suitable characteristic peak for monitoring the degradation of ampicillin trihydrate by FT-IR.

Time effect in stability test method was investigated for Ampi6 sample. The obtained results in Fig. 5 show that C=O band intensity decreases with increase in stability test time and degradation is complete after 48 h.

4. Conclusions

The influence of methylene chloride as a residual solvent is investigated on stability of ampicillin trihydrate crystal structure. Ampicillin trihydrate with high content of methylene chloride (>1500 ppm) is produced in an amorphous structure after stability test. Under high-temperature condition, the removal of methylene chloride molecules from ampicillin trihydrate cause defects in crystals. This phenomenon can be attributed to major degradation factor. The HPLC and FT-IR results show that degradation occurs at high level of methylene chloride. In this study, all the obtained results show that accelerated heat stability tests should be conducted to verify the expiry date given and residual solvents effects (especially the solvents having high limit) on pharmaceutical stability should be investigated.

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