Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis 36 (2005) 983–988
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

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

Saeed Nojavan[a], Alireza Ghassempour[b,*], Yosef Bashour[c], Masoud Khalilian Darbandi[c], Seyyed Hamid Ahmadi[d]

[a] *Department of Chemistry, Faculty of Science, Shahid Beheshti University, Tehran, Iran*
[b] *Medicinal Plants Research Institute, Shahid Beheshti University, Tehran, Iran*
[c] *Quality Control Lab., Zakaria Co., Tabriz, Iran*
[d] *Chemistry and Chemical Engineering Research Center of Iran, P.O. Box 14335-186, Tehran, Iran*

Received 30 May 2004; received in revised form 1 August 2004; accepted 27 August 2004
Available online 2 November 2004

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

* Corresponding author. Tel.: +98 21 29902681; fax: +98 21 2418679.
E-mail address: a-ghasempour@cc.sbu.ac.ir (A. Ghassempour).

0731-7085/$ – see front matter © 2004 Published by Elsevier B.V.
doi:10.1016/j.jpba.2004.08.031

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.