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Research Article

Synthesis and Characterization of Potential Impurity in Amoxicillin

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ABSTRACT

Amoxicillin is a potent bactericidal drug with activity against Gram-positive and Gram-negative bacteria. During the process development of amoxicillin, formation of various impurities was observed. Although, the structures and analytical procedures of these impurities have been already reported in the literature, surprisingly their synthesis was not accounted. In the present investigation, we have synthesized Amoxicillin impurity H namely (2*R*)-2-[(2,2-dimethylpropanoyl)amino]-2-(4-hydroxyphenyl) acetic acid.. The synthesized impurity has been characterized using FTIR, ^{1}H-NMR, Mass Spectrometry for m/z ratio and Elemental analysis.

KEYWORDS: Amoxicillin, Impurity H, Synthesis, Characterization.

INTRODUCTION

Amoxicillin, an acid stable, semi-synthetic drug, belongs to class of antibiotics called the Penicillins (β-lactam antibiotic). It is shown to be effective against the infections caused by wide range of Gram-positive and Gram-negative bacteria in both human and animals.[1-3] It is a congener of ampicillin (a semi-synthetic amino-penicillin) differing from the parent drug only by hydroxylation of phenyl side chain. It has found a niche in the treatment of ampicillin responsive infections after oral administration.[4-5] Chemically, amoxicillin is (2S,5R,6R)-6-[[(2R)-amino-2(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

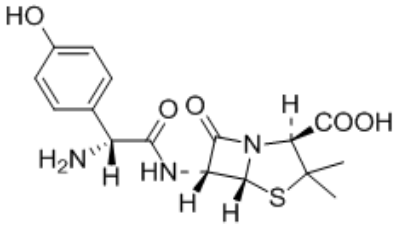


Fig. 1. Structure of Amoxicillin

Impurity removal is a critical and important task in pharmaceutical process research, where the final product meets stringent purity requirements. The presence of impurities in an active pharmaceutical ingredient (API) can have a significant impact on the quality and safety of the drug product. The guidelines recommended by ICH state that the acceptable levels for a known and unknown impurity in an API should be less than 0.15 and 0.10%, respectively.[6] These impurities are also required in pure form to understand the impurity profile and development of an accurate analytical method during the research and development phase.[7-9]

Several liquid chromatography (LC) methods have been reported for quantitative determination of amoxicillin hydrochloride and its related impurities in drug substance and drug products [10–16] dosage forms [17] and premixes.[18–19] Some LC methods with UV detection have been described for separation of side-chain diastereo isomers [20], C_5 epimers of amoxicilloic acids [21-22] and LC method with Mass Spectrometric detection for related substances has also been reported.[23] There have been a few attempts to study the detection of traces of degradation products of amoxicillin in environmental samples [24-26] and hospital sewage water. [27-28] At the time of development of amoxicillin, various process related impurities were observed. Literature reports include an increasing number of publications on the detection of impurities and the development of analytical methods for their analysis indicating the significance of impurities in amoxicillin. However, no synthetic details have been reported yet. In this context, in continuation to our previous work,[29] described for the identification, characterization and synthesis of amoxicillin impurities namely (4S)-2-[5-(4-hydroxyphenyl)-3, 6-dioxopiperazin-2-yl]-5,5-dimethyl thiazolidine-4-carboxylic acid (Amoxicillin Impurity C) and 3-(4-hydroxyphenyl) pyrazin-2-ol (Amoxicillin Impurity F), herein in the present study, we describe the identification, characterization and detailed experimental procedures for the synthesis of one more impurity of amoxicillin namely (2*R*)-2-[(2,2-dimethylpropanoyl) amino]-2-(4-hydroxyphenyl) acetic acid (Amoxicillin Impurity H). (Fig. 2).

MATERIALS AND METHODS

Samples of amoxicillin trihydrate and amoxicillin impurity H were obtained from the chemical research and analytical Department of Aurobindo Pharma, Hyderabad, India. The impurity was synthesized in the laboratory after

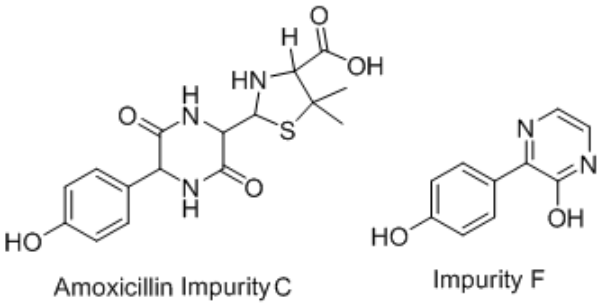


Fig. 2. Structures of Amoxicillin impurities C and F.

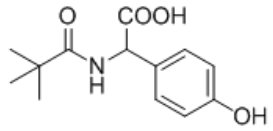
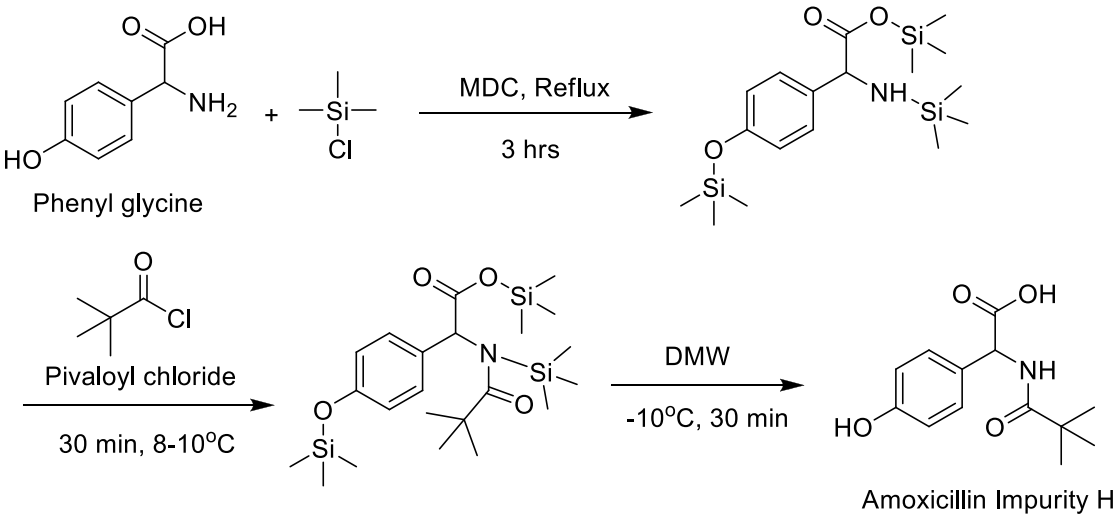


Fig. 3. Structure of Amoxicillin Impurity H



Scheme 1. Synthesis of Amoxicillin Impurity H

identification by HPLC and LC-MS. Acetonitrile (LC grade) from Merck (Germany) and Patassium dihydrogen orthophosphate (AR grade) from Rankem (Mumbai, India) were used. High purity water was prepared by use of a Millipore Milli Q plus (Milford, MA, USA) water-purification system.

High performance liquid chromatography (HPLC)

An Agilent HPLC system equipped with 1100 series low pressure quaternary gradient pump along with pulse dampener, Photo diode array detector with Autosampler has been used for the analysis of the sample. An Agilent Zorbax SB-C8, 150 mm × 4.6 mm, 5 µm column was employed for the testing of reaction mass of amoxicillin impurities. The column eluent was monitored at detection wavelength 230 nm. The mobile phase was 0.05 M potassium dihydrogen orthophosphate buffer; pH 5.0 (Mobile phase component A) and Acetonitrile (Mobile phase component B). Chromatography was performed with linear gradient program at flow rate of 1.5 ml/min. The column oven temperature was maintained at 40°C. Data was recorded by using Chemstation software.

NMR Spectroscopy: ^{1}H-NMR spectra of the compounds were recorded at a frequency of 400 MHz at 25°C on Bruker Avance II NMR Spectrophotometer, using TMS as an internal standard. The ^{1}H chemical shift values were reported on the δ scale in ppm, relative to TMS (δ = 0.0 ppm) and $CDCl_3$ (δ77.00 ppm).

FTIR Spectroscopy: The IR spectra were recorded in the solid state as a KBr dispersion medium using the FT-IR (Perkin Elmer, Spectrum Two) spectrophotometer.

Mass Spectroscopy: Molecular mass was determined by use of a Perkin Elmer API2000, PESCIEX triple quadrupole mass spectrometer with Analyst software.

RESULTS AND DISCUSSION

Synthesis of Amoxicillin impurity-H: A potential impurity of amoxicillin, which have not been reported previously. Amoxicillin Impurity H was synthesized by taking phenylglycine (10.0 g) and trimethylsilyl chloride (22.74 g) in 100 ml of methylene chloride at room temperature (22°C). The above solution was refluxed for 3 h. Further, the solution was cooled to 8-10^{0}C, pivaloyl chloride (10.8 g) was added to this solution and stirred for 30 minutes. After completion of the reaction, the temperature of the solution was maintained at -10^{0}C and water (50 ml) was added. The resulting solution was again stirred for 30 minutes at room

temperature. The aqueous layer was taken, the pH of the solution was adjusted to 3.1 with 50% HCl solution and again stirred for 2 h at room temperature. The precipitate formed was filtered, washed with water, air dried and finally recrystallized from 80% ethanol to give 5.7 g (65.4%) of crystalline (2*R*)-2-[(2,2-dimethylpropanoyl) amino]-2-(4-hydroxyphenyl) acetic acid (Impurity H). (Scheme-1)

Characterization: IR (KBr): λmax (cm^{-1}) at 3430, 3230, 3135, 2982, 1710, 1690, 1134; **^{1}H NMR:** 1.05 (s, 9H), 5.13-5.15 (d, 1H), 6.66-6.68 (m, 2H), 7.11-7.13 (m, 2H), 7.62-7.64 (d, 1H), 9.48 (s, 1H); **Mass Spectrum:** m/z 393 (M^+). **Elemental Analysis:** Calculated for $C_{13}H_{17}NO_4$: Calculated: C, 62.14; H, 6.82; N, 5.57; Found: C, 62.18; H, 6.72; N, 5.47%.

In summary, we have described a process to synthesize Amoxicillin Impurity H, a potential impurity of amoxicillin, which have not been reported previously. However, characterization and identification of degradation pathway of amoxicillin in this scheme is still a challenging task and require much more dedicated efforts in this direction. The mechanism of this reaction is still under study and believed to be produced by number of sequential steps. The synthesized impurity has been characterized using FTIR, ^{1}H-NMR, Mass Spectrometry for m/z ratio and Elemental analysis.

Keeping in mind the regulatory importance of amoxicillin impurities, our efforts to synthesize and characterize them effectively should prove to be valuable.

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