Research Article

Synthesis and Characterization of Potential Impurities 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 two impurities of Amoxicillin namely (4S)-2-[5-(4-hydroxyphenyl)-3, 6-dioxopiperazin-2-yl]-5, 5-dimethyl thiazolidine-4-carboxylic acid (Amoxicillin Diketopiperazine) and 3-(4-hydroxyphenyl) pyrazin-2-ol. The synthesized impurities have been characterized using FTIR, ^{1}H-NMR, Mass Spectrometry for m/z ratio and Elemental analysis.

Keywords: Amoxicillin, Characterization, Impurity C, Impurity F, Synthesis.

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.
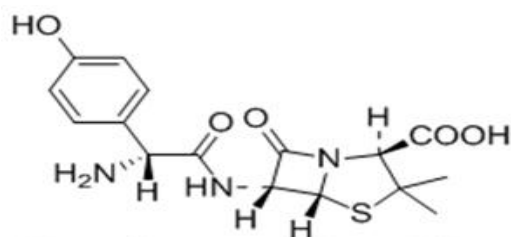

Fig. 1. Structure of Amoxicillin

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, the present study describes identification, characterization and detailed experimental procedures for the synthesis of amoxicillin impurity C and F. (Figure 2).
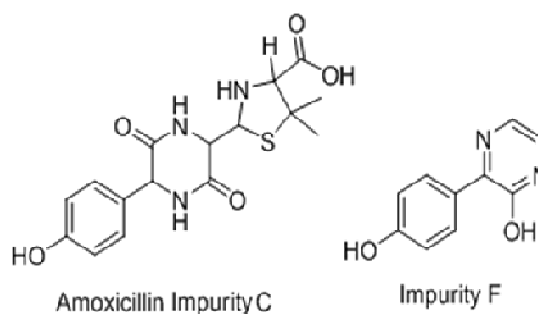

Fig. 2. Structures of Amoxicillin impurities C and F.

Experimental

Samples of amoxicillin trihydrate and its related substances were obtained from the chemical research and analytical department of Aurobindo Pharma, Hyderabad, India. All three impurities were synthesized in the laboratory after identification by HPLC and LC-MS. Acetonitrile (LC grade) from Merck (Germany) and Potassium 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 auto liquid sampler handling system 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 given in Table 1 at flow rate of 1.5 ml/min. The column oven temperature was maintained at $40^{o}C$. Data was recorded by using Chemstation software.

NMR Spectroscopy

1H-NMR spectra of the compounds were recorded at a frequency of 400 MHz at $25^{o}C$ on Bruker Avance II NMR Spectrophotometer, using TMS as an internal standard. The 1H 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.

Synthesis of Amoxicillin impurity-C

Amoxicillin Impurity C was synthesized by taking 10.0 g Amoxicillin in 100 ml of water at room temperature ($22^{o}C$) and pH of this solution was adjusted at 9.5±0.2 with 1.0 M NaOH solution. After adjustment of pH, the reaction solution was stirred for 60 min at room temperature. Further, the solution was heated to $50^{o}C$ and stirred for 5.0 h. After completion of the reaction, the pH of the solution was adjusted to 3.0 with 50% HCl solution and again stirred for 2 h at room temperature. The white precipitate formed was filtered, washed with

water, air dried and finally recrystallized from 80% ethanol to give 5.7 g (65.4%) of crystalline of Amoxicillin piperazine-2,5-dione (Impurity C).

Characterization IR (KBr)

λmax (cm^{-1}) at 3420, 3200-2900, 1735, 1660, 1610, 1595, 1518, 1450, 1270, 1195, 830; ^{1}H NMR: 1.25 (s, 3H), 1.60 (s, 3H), 3.62 (s, 1H), 3.81 (s, 1H), 4.88 (s, 1H), 5.121-5.128 (d, 1H), 6.74-6.76 (d, 2H), 7.11-7.13 (d, 2H), 7.44 (s, 1H), 8.47 (s, 1H), 9.25 (s, 1H); Mass Spectrum: m/z 393 (M^{+}). Elemental Analysis: Calculated for $C_{16}H_{19}N_3O_5S$; Calculated: C, 52.59; H, 5.24; N, 11.50; Found: C, 52.49; H, 5.14; N, 11.56 %.

Synthesis of Amoxicillin impurity F

Amoxicillin impurity F was synthesized by taking 20.0 g Amoxicillin in 1000 ml of water at room temperature ($22^{o}C$) and pH of this solution was adjusted at 3.1±0.2 with 50% HCl solution. The resulting solution was then heated to $75^{o}C$ with continuous stirring for 6.0 h. After completion of the reaction the solution was allowed to cool at room temperature. Extraction was done with 3X250 ml of chloroform; the organic layer was taken and dried over sodium sulphate. The crude product was collected by evaporating solvent under reduced pressure and purified by column chromatography using a mixture of Ethyl acetate and Hexane to obtain (3-(4-hydroxyphenyl) pyrazin-2-ol) (**Amoxicillin impurity-F**) to get 1.5 g of pure product.

Characterization of Amoxicillin Impurity F

IR (KBr) λmax (cm^{-1}) at 3200-2800, 1660, 1610, 1590, 1510, 1445, 1408, 1280, 1255, 1228, 1190, 830, 795; ^{1}HNMR: 6.80-6.78 (d, 2H), 7.18-7.17 (d, 1H), 7.36-7.35 (m, 2H), 8.25-8.22 (d, 2H), 9.59 (s, 1H). Mass Spectrum: m/z 393 (M^{+}); Elemental Analysis: Calculated for $C_{10}H_8N_2O_2$: Calculated: C, 63.82; H, 4.28; N, 14.89; O, 17.00; Found: C, 63.72; H, 4.23; N, 14.79.

RESULTS AND DISCUSSION

Chemistry

Amoxicillin Impurity-C

The in vitro formation and preparation of Ampicillin piperazine-2,5-dione was first described by Bundgaard and Larsen.[29] Roets et al.,[30] prepared amoxicillin piperazine-2,5-dione in a similar way. A number of papers [31-34] have described the epimerization of penicilloic acids, and indicated that the epimerization mainly occurs at the 5-position over a wide pH range between 2.5 to 13. Amoxicillin Impurity C was synthesized by taking amoxicillin in water at room temperature and pH of this solution was adjusted at 9.5±0.2 with 1M NaOH solution. After completion of the reaction the solution was stirred at room temperature. Further, the solution is heated for few hours and then the pH of the solution was adjusted to 3.0 with HCl solution (1:1) and again stirred for 2 hours at room temperature. The white precipitate formed was filtered, washed with water, air dried and finally

recrystallized from ethanol to give crystalline Amoxicillin piperazine-2,5-dione (Impurity C). (Scheme-1)

Scheme-1 Synthesis of impurity C
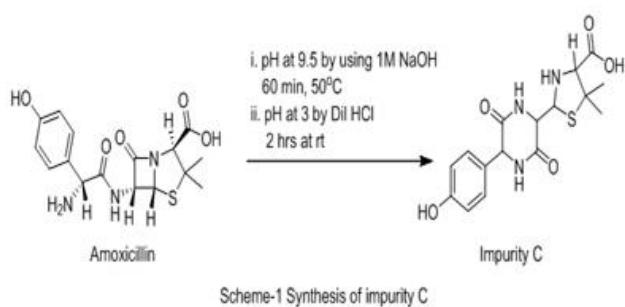
This method of obtaining the amoxicillin piperazine-2,5-dione appears to be a very simple procedure suitable for preparative purposes, the effect of pH on the yield was also studied. The increase in pH speeds up the reaction but the yield of the compound (Impurity C) was found to decrease with increasing pH at pH>10.0. At lower pH values the yield remained pH independent and therefore, a pH of about 9.5-10.0 is optimum as regards to the yield and time of reaction.

The degradation of amoxicillin **A** in acidic medium is expected to starts with the opening of the four membered β-lactam ring to give the penicilloic acid **B**, which contains a free carboxylic acid group and gives a higher polarity to this molecule. For the compound **2**, there are two possible pathways for further degradation. The first possible degradation reaction of intermediate **B** is the formation of a new, stable, six-membered ring giving diketopiperazine amoxicillin **C** (Amoxicillin impurity-C). The second one is the decarboxylation of the free carboxylic acid to give the stereoisomeric compounds amoxicillin penilloic acid I and II **D**. Both the reaction products were identified in the amoxicillin solution under acidic conditions. (Scheme-3). [23]
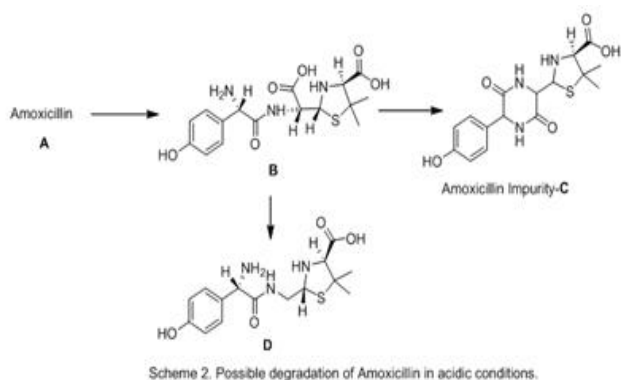
Scheme 2. Possible degradation of Amoxicillin in acidic conditions.

Amoxicillin Impurity F

A potential impurity of amoxicillin, which have not been reported previously. This impurity may be formed during the semi-synthetic preparation of these antibiotics or upon their degradation. Amoxicillin impurity F was synthesized by taking amoxicillin in water at room temperature and pH of this solution was adjusted at 3.1±0.2 with HCl solution (1:1). The resulting solution was then heated to 75°C with continuous stirring for 6 hours.

After completion of the reaction the solution was allowed to cool at room temperature and extraction with chloroform and the organic layer was taken and dried. The crude product was collected by evaporating the organic layer and purified by column chromatography using a mixture of Ethyl acetate and Hexane (Scheme-3).

Scheme-3 Synthesis of amoxicillin Impurity F
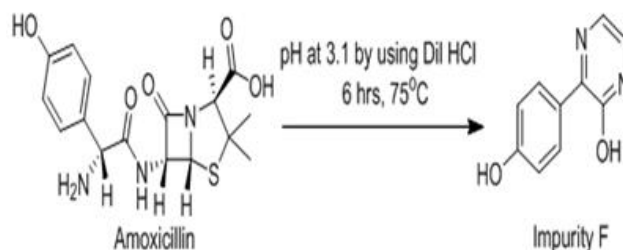
CONCLUSION

In summary, we have described a process to synthesize Amoxicillin Diketopiperazine (Amoxicillin Impurity C) which has many advantages over the already known method. For instance, this reaction offers advantages of an easy work-up, high yields, fast reaction rates etc. We moved a step forward to describe the possible degradation pathway of amoxicillin and investigation of the root cause of formation of Amoxicillin impurity C in proposed reaction conditions. In addition to this, we have also synthesized 3-(4-hydroxyphenyl) pyrazin-2-ol (Amoxicillin Impurity F), 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 impurities have 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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