ANALYSIS AND CHARACTERIZATION OF AMPICILLIN DEGRADATION PRODUCTS IN PHARMACEUTICAL SAMPLES BY LC-MS/MS

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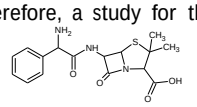
Introduction and Objectives

The consumption of antibiotics has grown dramatically in recent years, since they are not used exclusively as chemotherapeutic agents in humans [1]. However, the instability of beta-lactam antibiotics in solution was observed to be a major hurdle in the development of penicillin and other useful beta-lactam antibiotics.

The presence of such compounds in the environment indicates that they are recalcitrant and have low biodegradability. Therefore, antibiotics constitute an important class of drugs with potential to generate environmental impact due to its specific biological activity [2]. It is also crucial to characterize such degradation products because they might be present in medicines prescribed to infection treatments and the degradation would decrease the available amount of active principle. Therefore, a study for the characterization of antibiotic degradation products is justified by the need to solve such problems.

The main goal of this work was the determination and characterization of degradation products of ampicillin (AMP), a beta-lactam antibiotic, using liquid chromatography coupled to tandem mass spectrometry (LC-MS^n). For this purpose, the antibiotics standards were submitted to several treatments (from Brazilian regulation and procedures described in literature [3-5]), which induced their decomposition.


Ampicillin

Experimental Part

The analytical standard solutions were prepared and analyzed as shown aside. The analysis conditions were a chromatographic column KINETEX C18 – Phenomenex, 100 x 2.1 mm, 2.6 µm, 100 Å; mobile phases (MF) composed by 1% Formic Acid (aqueous MF - A) and Acetonitrile (organic MF - B) in a gradient as following (%B): 0-70% in 6 min, 70-30% in 11 min, 30-0% in 18 min and 0% until 27 min, with a flow of 0.25 mL min^{-1}. The equipment was an Agilent 1100 Series liquid chromatograph coupled to a Finnigan – LCQ DECA – ion trap mass spectrometer, using an electrospray (ESI) ionization source (positive mode). The MS conditions were capillary temperature 250°C, capillary voltage 11V; ESI voltage +4.5kV; Sheath gas flow 60 arbitrary units and Auxiliary gas flow 40 arbitrary units.

Ampicillin + (0.2 mol L^{-1} HCl or 0.2 mol L^{-1} NaOH or H_2O + heating at 60°C) → Analysis in t = 0h and t = 24hs

Results and Discussion

After analyses, it was possible to notice that the degradation products were the same comparing the three evaluated degradation methods. Basic degradation is effective within a short period of time while acid and heating treatments require a longer time to yield degradation products. However, they produced the same molecules and the possible routes, for analyses in t = 0h (Figure 1) and t = 24h (Figure 2), are shown bellow. The mass spectrum referent to the analysis right after sample preparation has other intense signal (*m/z* 698.80), since there was a dimmer formation, which was not observed after 24h (Figure 3). The chromatograms and spectra related to these analyses are presented in Figure 4.

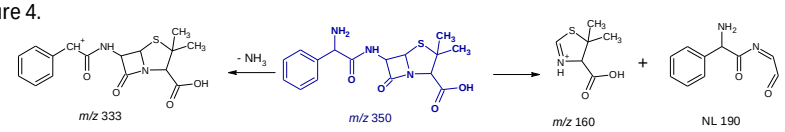


Figure 1. Degradation route of AMP for hydrolysis treatments in t = 0h.

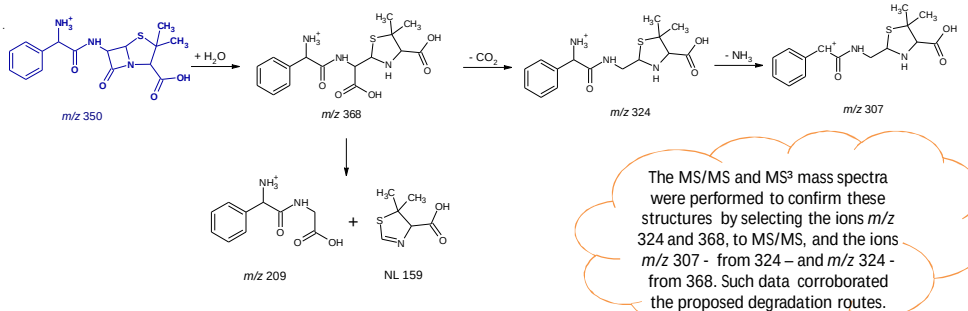


Figure 2. Degradation route of AMP for hydrolysis treatments in t = 24h.

The MS/MS and MS^3 mass spectra were performed to confirm these structures by selecting the ions *m/z* 324 and 368, to MS/MS, and the ions *m/z* 307 - from 324 – and *m/z* 324 - from 368. Such data corroborated the proposed degradation routes.

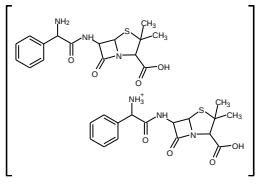


Figure 3. Dimmer formation of AMP in t = 0h.

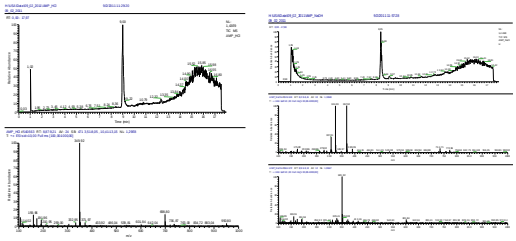


Figure 4. Chromatogram and mass spectra for analysis in t = 0h (left) and in t = 24h (right).

Conclusions

The proposal of degradation routes for the antibiotic ampicillin was performed after different treatments, such as acidic, basic and thermal degradation. In order to propose these routes, research in literature was proceeded and several degradation products were suggested based on the most probable fragmentations, according to the instability of the structures, as well as common reactions that occur in these degrading solutions. Therefore, the proposed pathways are consistent and they are in accordance with the obtained mass spectra.

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