

Detection of Ampicillin, its Sodium Salt and Alkaline Hydrolysis Products by MALDI-TOF Mass Spectrometry

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Abstract

Antibiotic resistance of bacteria is very big problem in clinical and veterinary medicine in recent year. This problem is increasing by the exposure of bacteria against antibiotics. The most spread of resistance is resistance against penicillins antibiotics. Ampicillin is common use antibiotic in the both medicine. Therefore, detection of antibiotics and antibiotic resistance are very important for prevention of spread of antibiotics in the environment and resistant bacteria too. Therefore the aim of this study was detection of ampicillin, its sodium salts and alkaline hydrolysis products by MALDI-TOF Mass Spectrometry. For detection of pure ampicillin, its sodium salts and alkaline hydrolysed products MALDI-TOF MS (Matrix Assisted Light Desorption Ionization – Time of Flight Mass Spectrometry) Microflex LT in positive ion mode was used. Determined mass spectra were observed by FlexAnalysis software. The mass spectra and molecular weight of ampicillin, its sodium salts and hydrolysed products were compared with theoretical molecular weight of these compounds. The results showed that it is possible to detect ampicillin, its sodium salts and hydrolyzed products by MALDI-TOF MS in positive ion mode with some correction in analytical software. This knowledge is resulting that MALDI-TOF MS detection method can be useful for detection of ampicillin from different kind of environment samples and it is possible to detect ampicillin resistance mechanism (enzymatic destruction) indirectly, because resistance against penicillins is enzymatic hydrolysis of beta-lactam core. Also the most important issue is that method is very quickly and cheap.

Keywords: Detection, ampicillin, hydrolysis, MALDI-TOF MS

1. Introduction

In recent years, antibiotics have been recognized as the emerging environmental pollutants in aquatic environments, because of their potential adverse effects on the ecosystem and human health [1,2]. Large amounts of antibiotics and their metabolites are potentially released into aquatic environment, which are responsible for the selection of antibiotic resistance in bacteria pathogens [3,4]. Nowadays, many studies showed that the bacteria were resistant to several antibiotics and are capable of transferring their

resistant determinants among different genera in different environment [5-8]. Therefore, accurately monitoring trace amounts of antibiotics in different kind of environment is necessary and urgent. Up to now, many analytical methods have been reported for the determination of antibiotics, such as fluorescence detection [9], high performance liquid chromatography (HPLC) [10], capillary electrophoresis [11], enzyme-linked immunosorbent assay (ELISA) [12], surface plasmon resonance (SPR) [13].

β -Lactams, the antibiotics most frequently used to treat bacterial infections diseases [14] are becoming less useful against enterobacteria. The principal cause of this decrease in efficacy is the production of extended-spectrum β -lactamases (ESBLs) by these bacteria [14]. Mass

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spectrometry methods, such as MALDI-TOF, detect mass shifts in the molecule we are following-up. To detect bacteria having beta-lactamase enzymes by MALDI-TOF, the mass spectra of the antibiotic can be monitored, observing the disappearance of the original mass peaks and the appearance of new ones, corresponding to the hydrolyzed products as a new structure is originated [15–17]. If bacteria are sensitive, the antibiotic spectra will not change. Appearance of the mass peak corresponding to the beta-lactamase enzyme can be monitored, but it has only been described for ampicillin-resistant bacteria [18] and for the identification of CMY-2 b-lactamases [19]. Besides, these methods that focus on the enzyme identification are much slower and more tedious to carry out in a clinical laboratory. MALDI-TOF MS is routinely and satisfactorily applied to identify bacteria in clinical microbiology laboratories. However, it has recently been used to detect microorganisms harboring specific antimicrobial resistance mechanisms [20]. Therefore the aim of this study was to observation of ampicillin, its sodium salt, hydrolyzed ampicillin and its sodium salts produced by alkaline hydrolysis with using sodium hydroxide.

2. Materials and methods

Chemicals and preparation

In this study we measured ampicillin, its sodium salt and hydrolyzed products. Ampicillin was dissolved in pure H₂O and sodium hydroxide (100mM) was added to AMP+water solution to equal final concentration 10 mM. Concentration of ampicillin started from 1 mg/mL to 20µg/mL. The all chemicals were obtained from Sigma Aldrich, Germany and it were the mass spectrometry purity. As matrix α -hydroxy-4-cinnamic acid (HCCA) (Bruker Daltonics, Germany) for MALDI-TOF Mass Spectrometry was used.

MALDI-TOF MS analysis of ampicillin

Matrix (HCCA) (10 mg/mL) was dissolved in 250µL of organic solution which was prepared from 50µL of 100% acetonitril (Sigma Aldrich, Germany), 47,5µL pure distilled water (Sigma Aldrich, Germany) and 25µL of 100% trifluoroacetic acid (Sigma Aldrich, Germany). All chemicals used for preparing of organic solution was in MS purity. A 1µL of ampicillin solutions (without and with NaOH) were transferred to the MALDI-TOF microplate (Bruker Daltonics, Germany) and after drying covered with matrix solution. Mass spectra were obtained using MALDI-TOF MS Microflex LT (Bruker Daltonics, Germany) working in linear positive ion mode ranging from 360 to 600 m/z. Determined spectra were analysed by software flexControl 3.0 (Bruker Daltonics, Germany). Parameters of MALDI-TOF MS were set as follow: source ion 1, 20kV; source ion 2, 16.2kV; lens 7 kV; pulsed ion extraction 170 ns; detection gain 3.0x; electronic gain, regular; mode, low range; mass range selection, low range; laser frequency, 60Hz; digitizer trigger level, 2500 mV; laser attenuator, 24%, laser attenuator 30 % and laser range from 70 to 90%. Spectra were measured randomly at least 5 position.

Alkaline ampicillin hydrolysis

A 100 mM NaOH (100µL) was added to 900µL ampicillin + water solution. Solution was mixed by shaking. Its common knows that ampicillin can be hydrolyzed in alkaline environment. For alkaline hydrolysis NaOH was used in this experiment. Ampicillin molecule has 349m/z and ampicillin sodium salt 371m/z (Fig. 1). Sodium molecular weight is about 22m/z. Alkaline hydrolysis of ampicillin is similar as enzymatic hydrolysis by beta-lactamase (Fig. 2), which produce penicillin resistant bacteria.

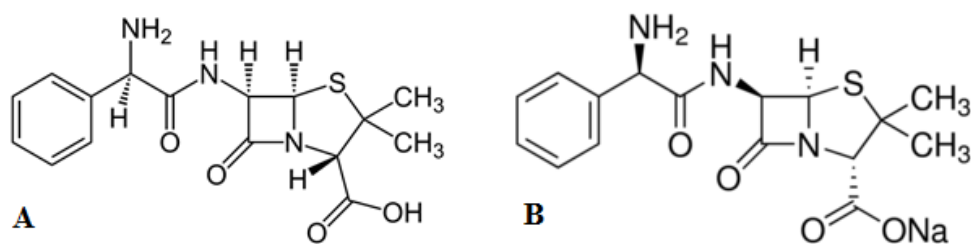


Figure 1. Molecules of ampicillin (A) and ampicillin sodium salt (B)

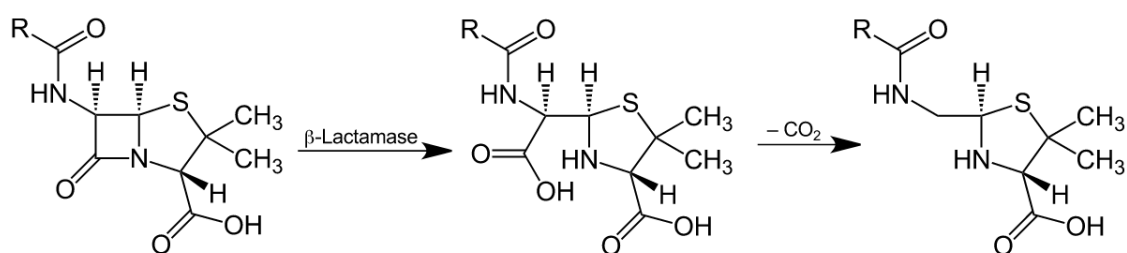


Figure 2. Hydrolysis of ampicillin by beta-lactamase and spontaneous decarboxylation

Analysis of spectra

Obtained spectra were analyzed with using flexAnalysis 3.0 software (Bruker Daltonics, Germany). Peaks were detected by centroid detection algorithm with a signal-to-noise threshold of 1, a relative intensity threshold of 0%, a minimum intensity threshold of 0, a peak width of 0.2m/z, a height of 80%, a TopHat baseline subtraction, smoothing with the Savitzky-Golay algorithm, a width of 0.2m/z, and a cycle of 1. Detected peaks were compared with the theoretical molecular masses of ampicillin, its sodium salt and hydrolysed, decarboxyated ampicillin and its sodium salts, with a ± 0.6 m/z tolerance.

Verification and internal calibration of MALDI-TOF MS

The peaks of ampicillin and its sodium salt, matrix (HCCA) were used for internal calibration of MALDI-TOF mass spectrometer.

3. Results and discussion

Matrix HCCA was used in this experiment. The spectra of pure HCCA matrix were identified in 189m/z and 378m/z and it were obtained using 10 mg/mL of α -hydroxy-4-cinnamic acid (HCCA) in pure distilled H₂O for mass spectrometry. Also

native pure ampicillin (348.373m/z) and its sodium salt (371.000m/z) were observed (Fig. 3A). The minimal concentration of ampicillin able to observation by MALDI-TOF MS were determined at 20 μ g/mL. Hrabák et al. [17] observed meropenem in native form and its hydrolyzed products with using MALDI-TOF MS. They dissolved other matrix 2.5-dihydroxybenzoic acid (DHB) in 50% ethanol and they observed meropenem and its sodium salts. Also, they determined the lowest detection limiting concentration for meropenem detection (19.173mg/L) by MALDI-TOF MS. The modification of the ampicillin molecule produced by beta-lactamases is theoretically similar to that produced by hydrolysis of NaOH. Alkaline hydrolysis by NaOH produced several following products: hydrolyzed ampicillin (366.735m/z), hydrolyzed ampicillin sodium salt (389.340m/z), hydrolyzed ampicillin disodium salt (412.254m/z). Equally, hydrolyzed ampicillin subject to spontaneous decarboxylation and its produced hydrolyzed decarboxyated ampicillin (323.958m/z) and hydrolyzed decarboxyated ampicillin sodium salt (344.253m/z) (Fig. 3B). Sparbier et al. [18] observed different type of antibiotics which can be hydrolyzed by NaOH and beta-lactamases and they determined following mass spectra for ampicillin and its sodium salt and hydrolyzed products: ampicillin + H (350.1m/z), ampicillin

sodium salt (372.1m/z), ampicillin disodium salt (394.1m/z), hydrolyzed ampicillin (367.9m/z), hydrolyzed ampicillin sodium salt (389.9m/z), hydrolyzed ampicillin disodium salt (411.9m/z)

and decarboxylated hydrolyzed ampicillin (324.0m/z). In contrast with their results we observed decarboxylated hydrolyzed ampicillin sodium salt (344.253m/z).

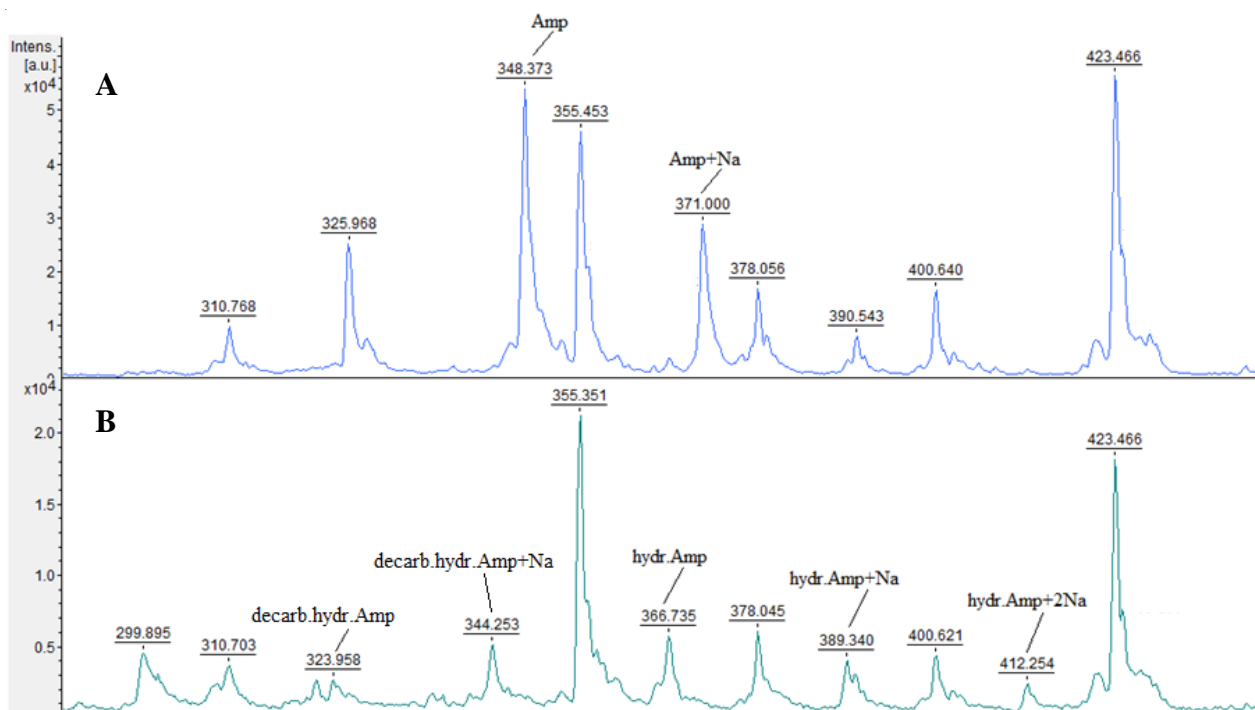


Figure 3. Spectral analysis of ampicillin, its sodium salts (A), hydrolyzed ampicillin, its sodium salts, decarboxylated hydrolysed ampicillin and its sodium salt (B)

Legend: Amp – ampicillin, Amp+Na – ampicillin sodium salt, decarb. hydr. Amp – decarboxylated hydrolyzed ampicillin, decarb. hydr. Amp+Na – decarboxylated hydrolyzed ampicillin sodium salt, hydr. Amp – hydrolyzed ampicillin, hydr. Amp+Na – hydrolyzed ampicillin sodium salt, hydr. Amp+2Na – hydrolyzed ampicillin disodium salt

4. Conclusions

Our results showed that Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry can be used for detection of ampicillin, its sodium salts, hydrolyzed products and its sodium salts with high precision. MALDI-TOF MS can to differentiate molecules with high precision and it can be very useful tool for detection of small molecule as antibiotics. The basic matrix as HCCA can be used for ampicillin detection and MALDI-TOF MS can to observe peaks of ampicillin and its products in low concentration. Also alkaline hydrolysis of ampicillin can be used for detection of beta-lactamases in penicillins resistant bacterial strains, but no directly with measure of beta-lactamases but indirectly with measure of breakdown products of ampicillin. Equally, this method can be used for detection of ampicillin in different

types of environment where ampicillin can be present.

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