REACTION OF AMPICILLIN AND AMOXICILLIN WITH ALCOHOLS

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ABSTRACT

HPLC/ESI-MS analyses of alcohol solutions of ampicillin and amoxicillin have shown that the two drugs react very readily with alcohols (methanol and ethanol) at room temperatures, without the presence of any catalyst. Products of methanolysis were detected even when methanol was used as a mobile phase, in HPLC/ESI-MS analysis of a water solution of ampicillin and amoxicillin. Therefore, neither methanol nor other alcohols should be used when working with these compounds.

Keywords: ampicillin, amoxicillin; β-lactam antibiotics; HPLC/ESI-MS; methanol; alcohol

INTRODUCTION

Hydrolysis of β-lactam ring leads to deactivation of β-lactam antibiotics, e.g., ampicillin or amoxicillin. Hydrolysis of these compounds is catalyzed by their respective enzymes, mainly Zn-β-lactamases [1-9], by enzyme analogues [10, 11] and may also occur in non-enzymatic media, especially under basic conditions [12-14]. Beside hydrolysis, methanolysis of β-lactam antibiotics has also been widely studied, mainly in the presence of metal ions [15-20], rarely in enzymatic media [21]. Ampicillin and amoxicillin are two common β-lactam antibiotics, so obviously their hydrolysis and methanolysis have been studied as well [6, 8, 12-14, 17-19]. However, hydrolysis of ampicillin and amoxicillin will not occur in neutral water solutions, even for several days [22, 23].

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In this paper it is shown that ampicillin and amoxicillin react very readily with alcohols at room temperatures, therefore, the use of methanol as a solvent when working with these drugs is not recommended.

MATERIALS/METHODS/PROCEDURES

Ampicillin and amoxicillin were obtained from Sigma-Aldrich (Poznań, Poland).

The HPLC/ESI-MS analyses were performed using a Waters model 2690 HPLC pump (Milford, MA, USA), a Waters/Micromass ZQ2000 mass spectrometer (single quadrupole type instrument equipped with electrospray ion source, Z-spray, Manchester, UK). The software used was MassLynx V3.5 (Manchester, UK). The drug concentrations in the sample solutions were 10^{-5} mol/dm^3. Using an autosampler, the sample solutions were injected onto the XBridge C18 column (3.5 μm, 100 mm x 2.1 mm, i.d., Waters). The injection volume was 10 μl.

The alcohol sample solutions were analyzed using a linear gradient of CH_3CN–H_2O with a flow rate of 0.2 ml/min. The gradient started from 0% CH_3CN – 95% H_2O with 5% of a 10% solution of formic acid in water, reaching 95% CH_3CN after 15 min, and the latter concentration was maintained for 5 min. The water solutions were analyzed at isocratic conditions, as shown in Figure 5, also with the flow rate of 0.2 ml/min.

Mass spectra were recorded in the m/z range 100-1000, in positive and negative modes simultaneously (during the HPLC/ESI-MS analyses, the mass spectrometer was switched in the fast mode between the positive and negative ion modes). The electrospray source potentials were: capillary 3 kV, lens 0.5 kV, extractor 4 V, and cone voltage 30 V. The source temperature was 120°C and a desolvation temperature of 300°C. Nitrogen was used as the nebulising and desolvation gas at the flow rates of 100 and 300 l h^{-1}, respectively.

RESULTS AND DISCUSSION

HPLC/ESI-MS analysis of methanol (or ethanol) solutions of ampicillin (**1**) or amoxicillin (**2**) revealed that the reaction leads to the formation of compounds **1a** and **2a** – products of methanolysis, as well as **1b** and **2b** – products of ethanolysis (Scheme 1, there are no other logical alcoholysis products having the same masses).

A detailed presentation of each of the HPLC/ESI-MS analyses performed does not seem to be necessary. To keep this paper within reasonable length, only representative examples are shown for ampicillin, though analogous results were obtained for amoxicillin.

Figure 1 shows ESI mass spectra of **1**, **1a**, **1b** (in both positive and negative ion modes) obtained upon HPLC/ESI-MS analysis of the respective ampicillin solutions, and Figure 2 shows ESI mass spectra obtained for amoxicillin solutions (**2**, **2a**, **2b**).
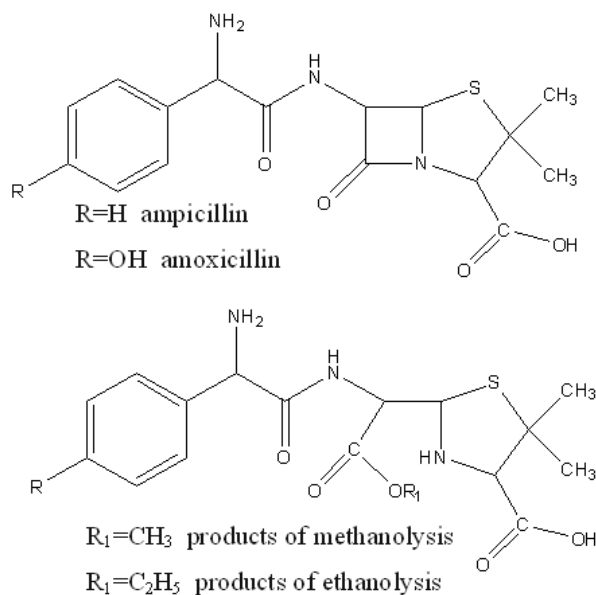
Scheme 1. Structures of ampicillin (**1**, R=H), amoxicillin (**2**, R=OH) and the products of their alcoholysis (**1a** R=H R$_1$=CH$_3$ and **2a** R=OH R$_1$=CH$_3$ – products of methanolysis; **1b** R=H R$_1$=C$_2$H$_5$ and **2b** R=OH R$_1$=C$_2$H$_5$ – products of ethanolysis)

Although ESI is a very "soft" ionization method (fragmentation does not usually occur), a mass spectrometric decomposition of products of alcoholysis is observed (Figures 1 and 2). A detailed MS fragmentation study is not the purpose of this paper. Obviously, most important are the ions corresponding to the protonated and deprotonated molecules. It is worth adding that a mass spectrometric decomposition of hydrolysis products of ampicillin and amoxicillin also occurs very readily under ESI conditions [24, 25].

Figure 3 shows single ion chromatograms of ions [**1**+H]$^+$ (m/z 350) and [**1a**+H]$^+$ (m/z 382) obtained upon HPLC/ESI-MS analyses of methanol solutions of ampicillin. It is clearly seen that, even after a few minutes, a significant amount of **1a** is formed from ampicillin being dissolved in methanol and the amount of **1a** is much higher after a few hours, compared with **1**. Analogous results were obtained for amoxicillin. Figure 4 shows chromatograms obtained for the ethanol solution of ampicillin in the negative ion mode. Similarly as for methanolysis, the ethanolysis of ampicillin occurs very readily.
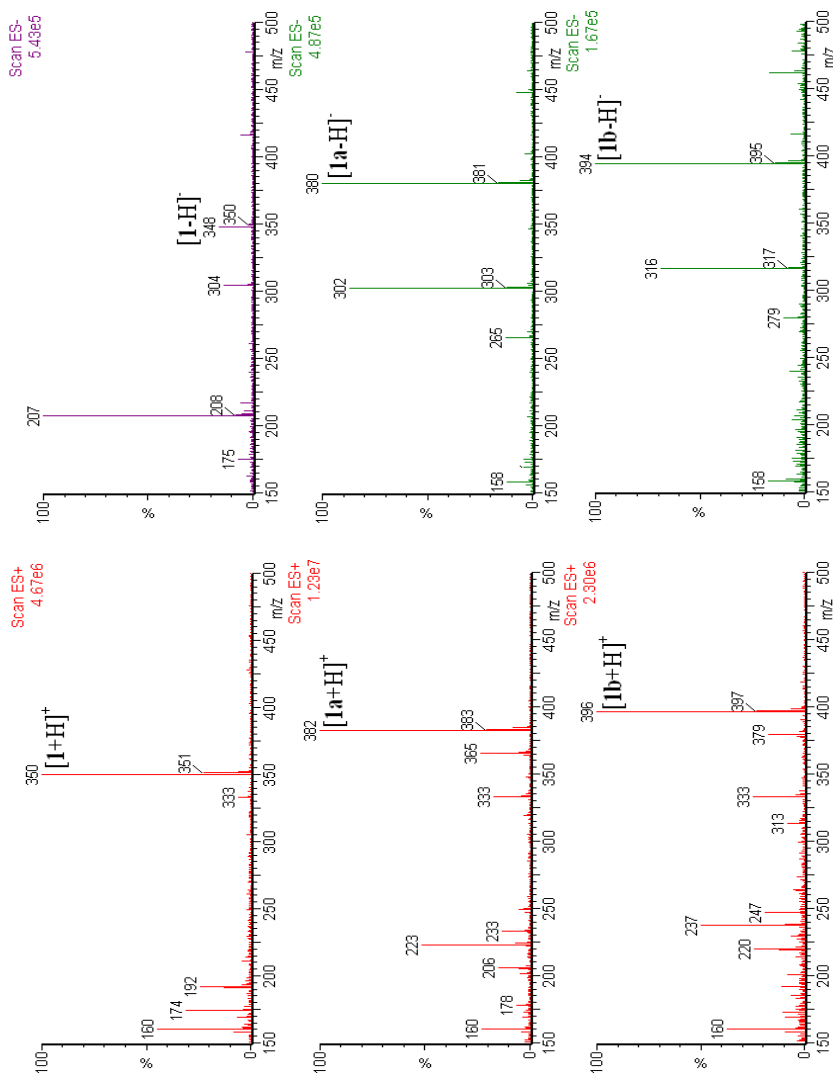
Fig. 1.   Full scan ESI mass spectra of ampicillin (**1**) and the product of its methanolysis (**1a**) and ethanolysis (**1b**). The spectra were obtained upon HPLC/ESI-MS analysis performed in both the positive and negative ion modes. Although ESI is a very "soft" ionization method (fragmentation does not usually occur), the mass spectrometric decomposition of products of alcoholysis is observed.
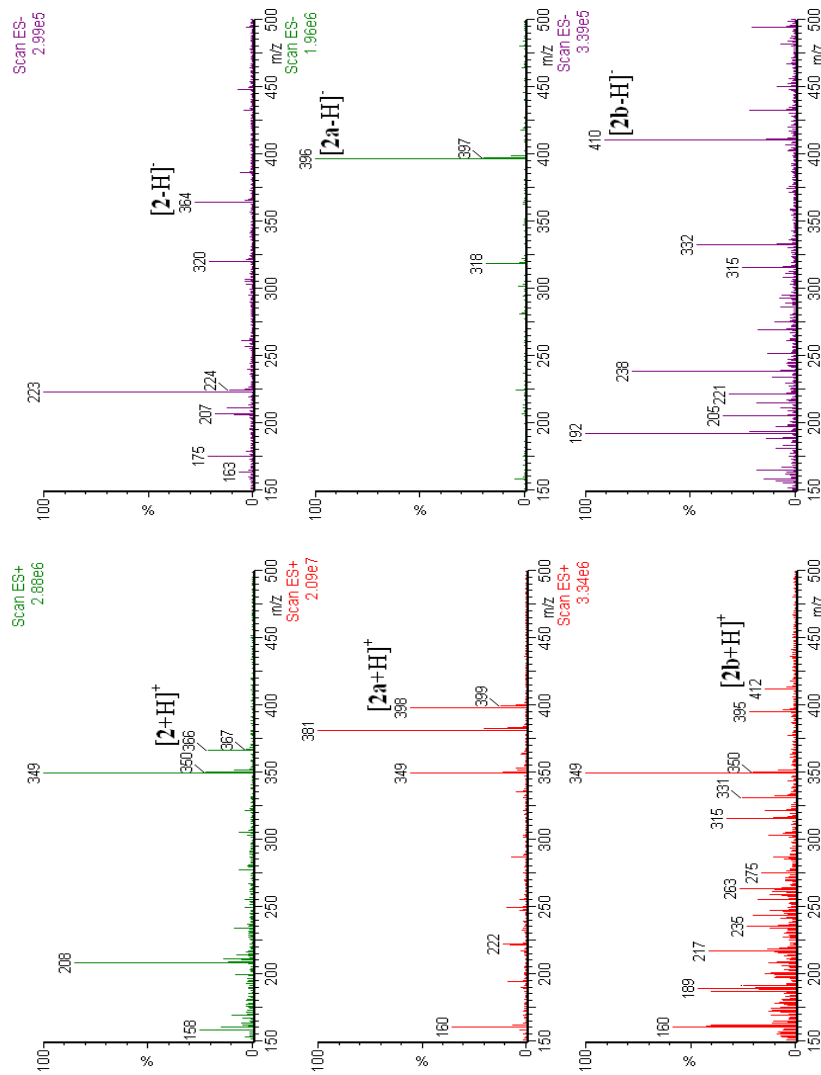
Fig. 2.   Full scan ESI mass spectra of amoxicillin (**2**) and the product of its methanolysis (**2a**) and ethanolysis (**2b**). The spectra were obtained upon HPLC/ESI-MS analysis performed in both the positive and negative ion modes.
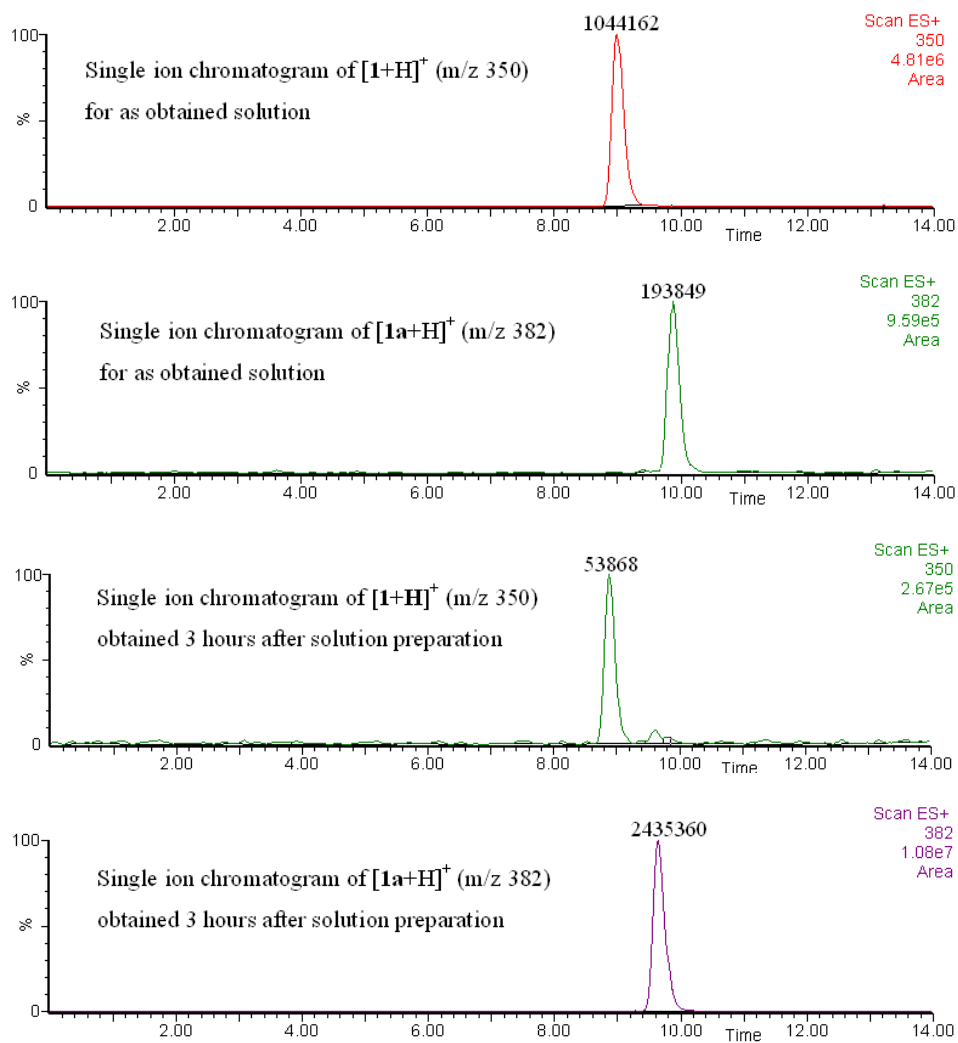
Fig. 3. Single ion chromatograms of ions [**1**+H]$^+$ (m/z 350) and [**1a**+H]$^+$ (m/z 382) ob-
tained upon HPLC/ESI-MS analyses of methanol solutions of ampicillin. Chro-
matographic peaks are assigned by peak areas (in arbitrary units).

Fig. 4. Single ion chromatograms (SIC) of ions [1-H]⁻ (m/z 348) and [1b-H]⁻ (m/z 394) obtained upon HPLC/ESI-MS analyses of ethanol solutions of ampicillin. Chromatogr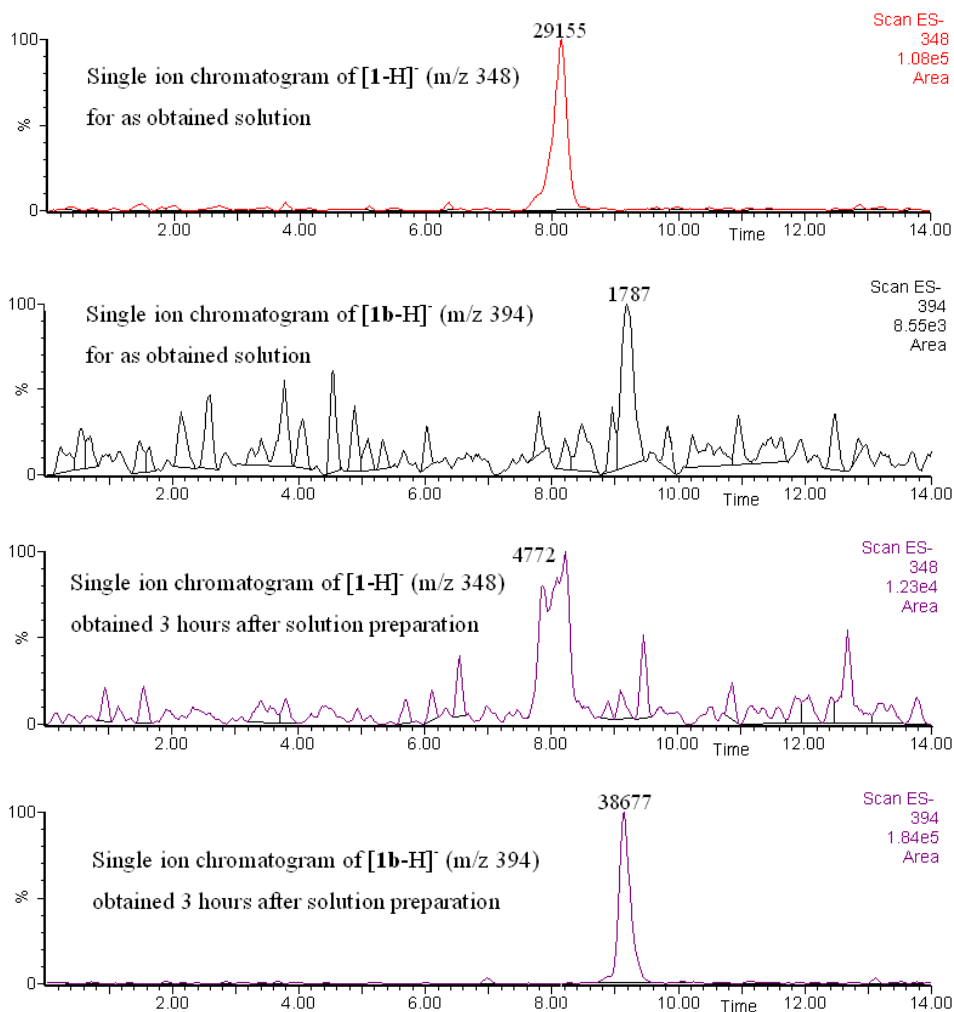aphic peaks are assigned by peak areas (in arbitrary units). It is clear that, similarly as for methanolysis, ethanolysis of ampicillin occurs very readily.

Methanol is a common solvent used as a mobile phase in liquid chromatography. It was found that the reaction between methanol and ampicillin (or amoxicillin) may occur even when methanol is used as a mobile phase, during HPLC analysis. Figure 5 shows single ion chromatograms of the ions $[1+H]^+$ (m/z 350) and $[1a+H]^+$ (m/z 382) obtained upon HPLC/ESI-MS analyses of water solutions of ampicillin by using different mobile phase compositions, at isocratic conditions. When the mobile phase contained no methanol, compound **1a** was not detected. However, for the mobile phase containing methanol, compound **1a** was detected.
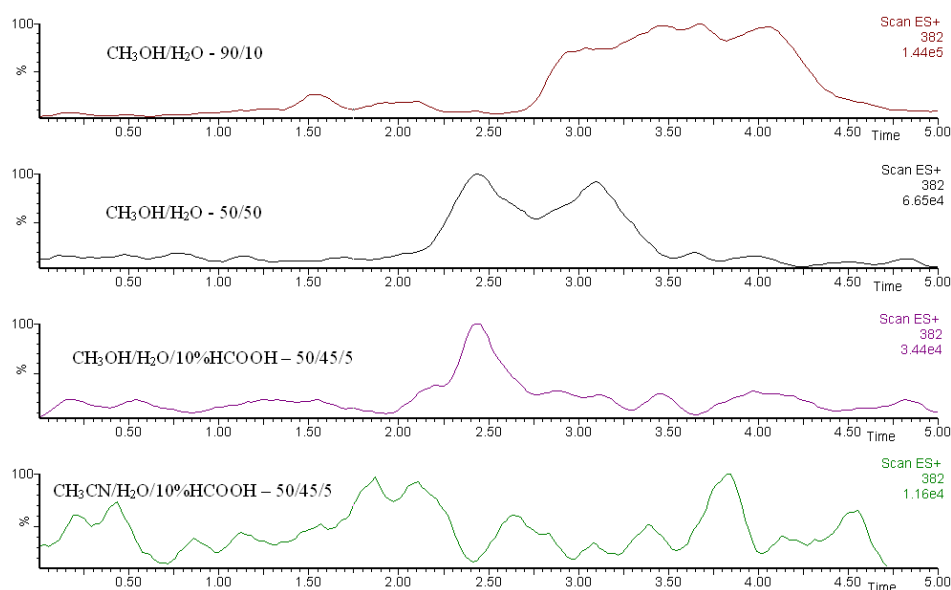

Fig. 5.   Single ion chromatograms of $[1a+H]^+$ (m/z 382) obtained upon HPLC/ESI-MS analysis of ampicillin water solution at isocratic conditions (the conditions are given at the chromatograms).

From the point of view of separation, these chromatographic conditions are not good (chromatographic peaks are broad) but for the purpose of this work the conditions provide satisfactory results.

It is an established fact that methanolysis of β-lactam antibiotics is more effective under basic conditions than under neutral conditions (however, the mobile phase basification for HPLC/ESI-MS is not a common practice, as it may cause apparatus contamination). If pH increase leads to a higher efficiency of methanolysis, its decrease should yield its lower efficiency. Therefore, the amount of **1a** formed under acidic conditions, $CH_3OH/H_2O/10\%HCOOH$ – 50/45/5, was much lower than under neutral conditions, CH_3OH/H_2O – 50/50 (Figure 3). It is worth noting that acidic conditions lead to a higher response of protonated molecules. Obviously, as expected, the most intense signal of **1a** was

observed for the mobile phase containing the highest amount of methanol, CH_3OH/H_2O – 90/10 (Figure 5).

CONCLUSION

The conclusion of this communication is that methanol should not be used as a solvent during analysis of ampicillin and amoxicillin, even though there are a number of examples of the use of methanol in working with these compounds [26-34]. Obviously, this paper is not meant, in any way, to debase [26-34] or other papers, however, it is a generally accepted rule in chemistry that the solvent used should not react with the compounds analyzed.

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