Comparison of the ESI responses of penicillins with ESI responses of their methanolysis products - **supplementary material**

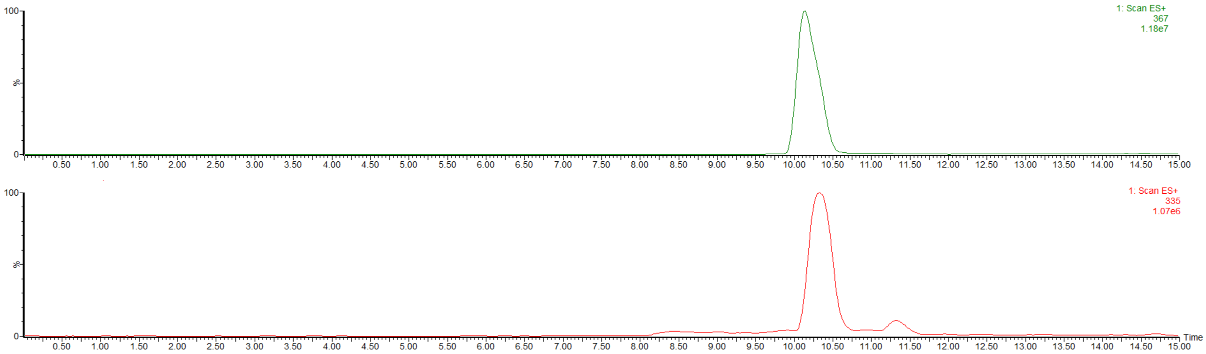

Figure 1s. Exemplary single ion chromatograms of [**Pen**$_{\mathrm{methanolyzed}}$+H]$^+$ (m/z 367, top) and [**Pen**+H]$^+$ (m/z 335, bottom) obtained upon HPLC/ESI-MS analysis. It is clearly seen that penicillin and it methanolysis product are not separated (they overlap). However, even when column of 30 mm long is was they separation was also poor (Amelin & Avdeeva J. Anal. Chem. 2018; 73: 922-928).

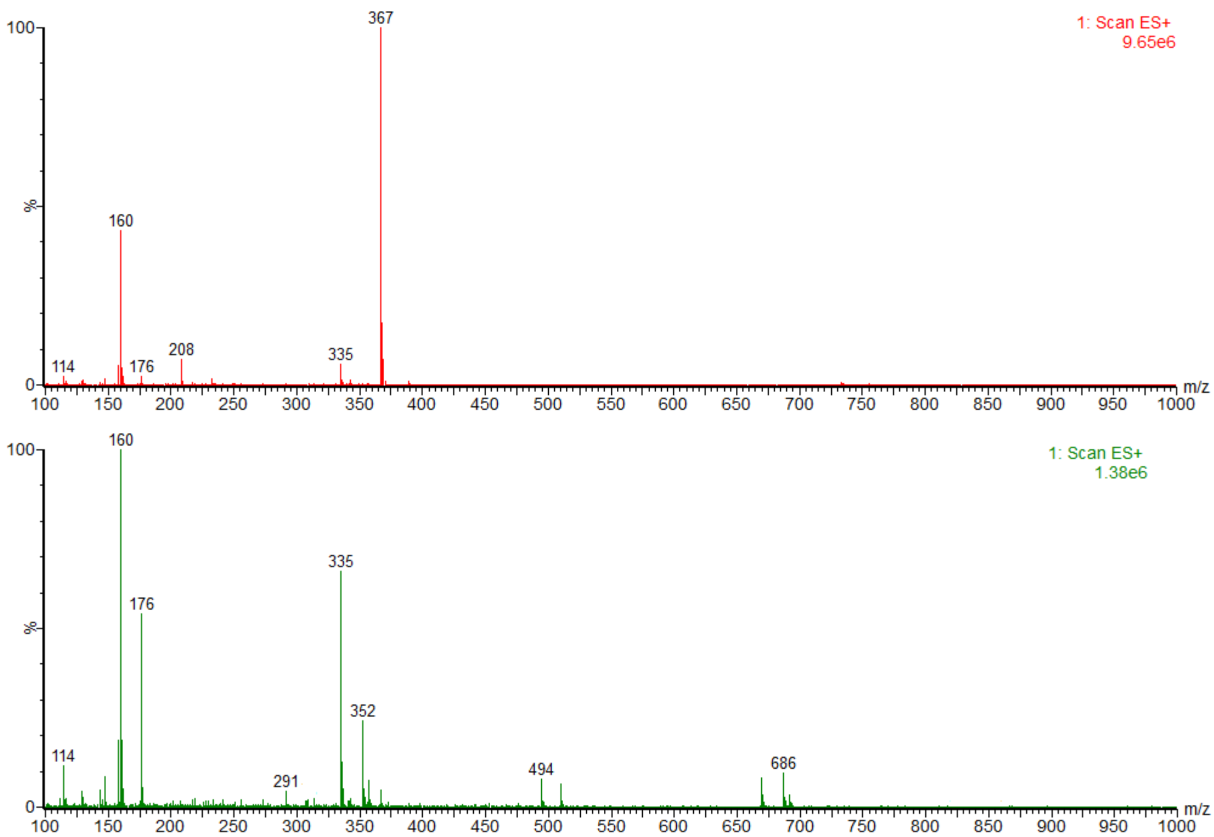

Figure 2s. Exemplary full scan mass spectra of penicillin methanolysis product (top) and penicillin (bottom) obtained upon HPLC/ESI-MS analysis.

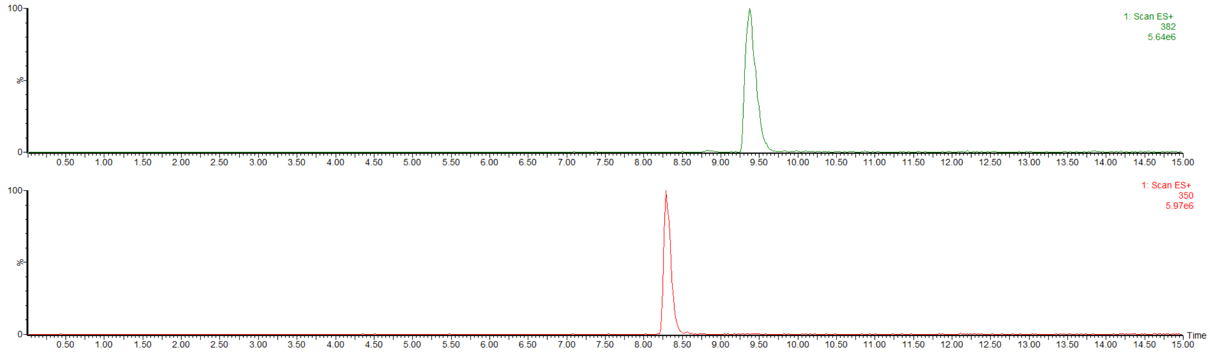

Figure 3s. Exemplary single ion chromatograms of [**Amp**$_{methanolyzed}$+H]$^+$ (m/z 382, top) and [**Amp**+H]$^+$ (m/z 350, bottom) obtained upon HPLC/ESI-MS analysis.

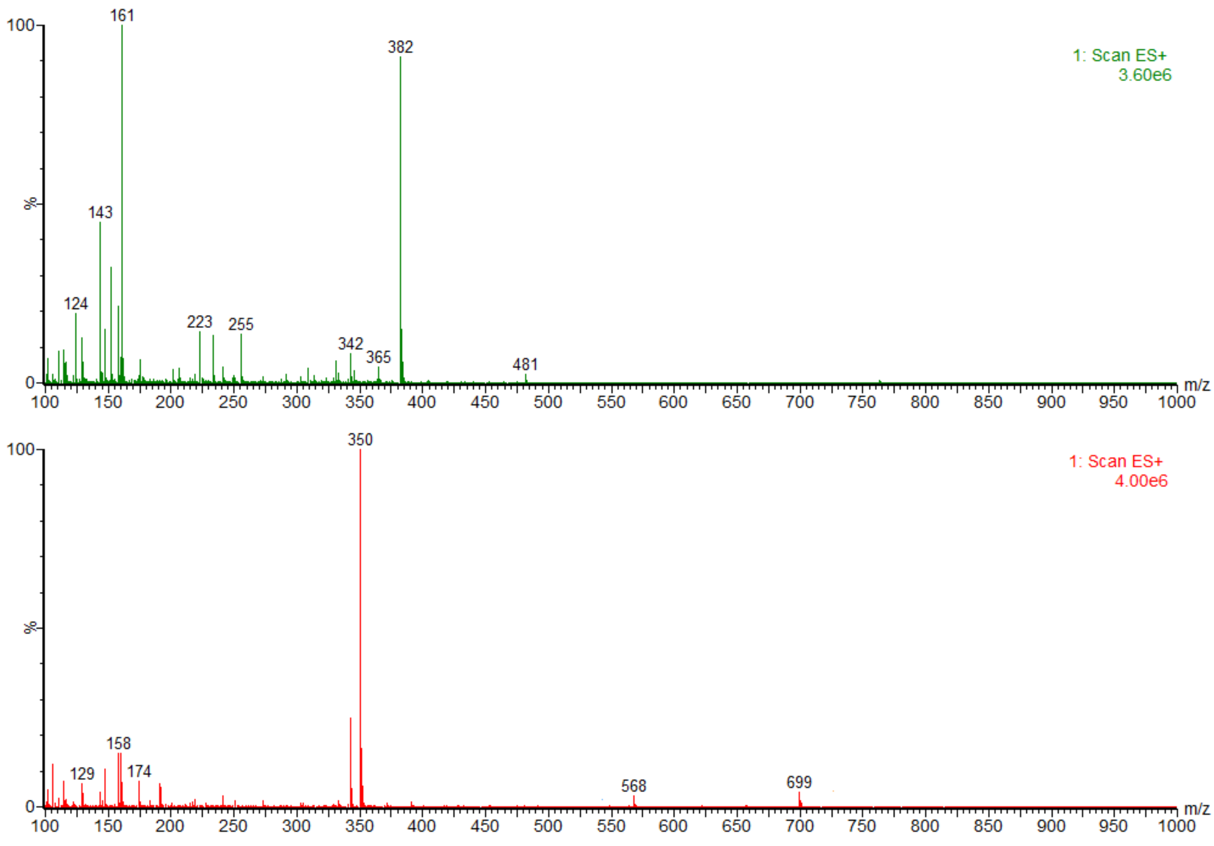

Figure 4s. Exemplary full scan mass spectra of ampicillin methanolysis product (top) and ampicillin (bottom) obtained upon HPLC/ESI-MS analysis.

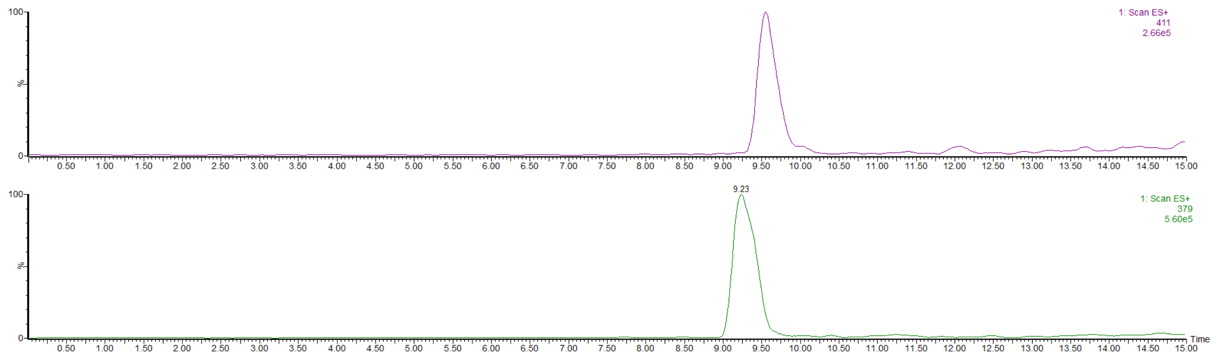

Figure 5s. Exemplary single ion chromatograms of [**Carb**$_{methanolyzed}$+H]$^{+}$ (m/z 411, top) and [**Carb**+H]$^{+}$ (m/z 379, bottom) obtained upon HPLC/ESI-MS analysis.

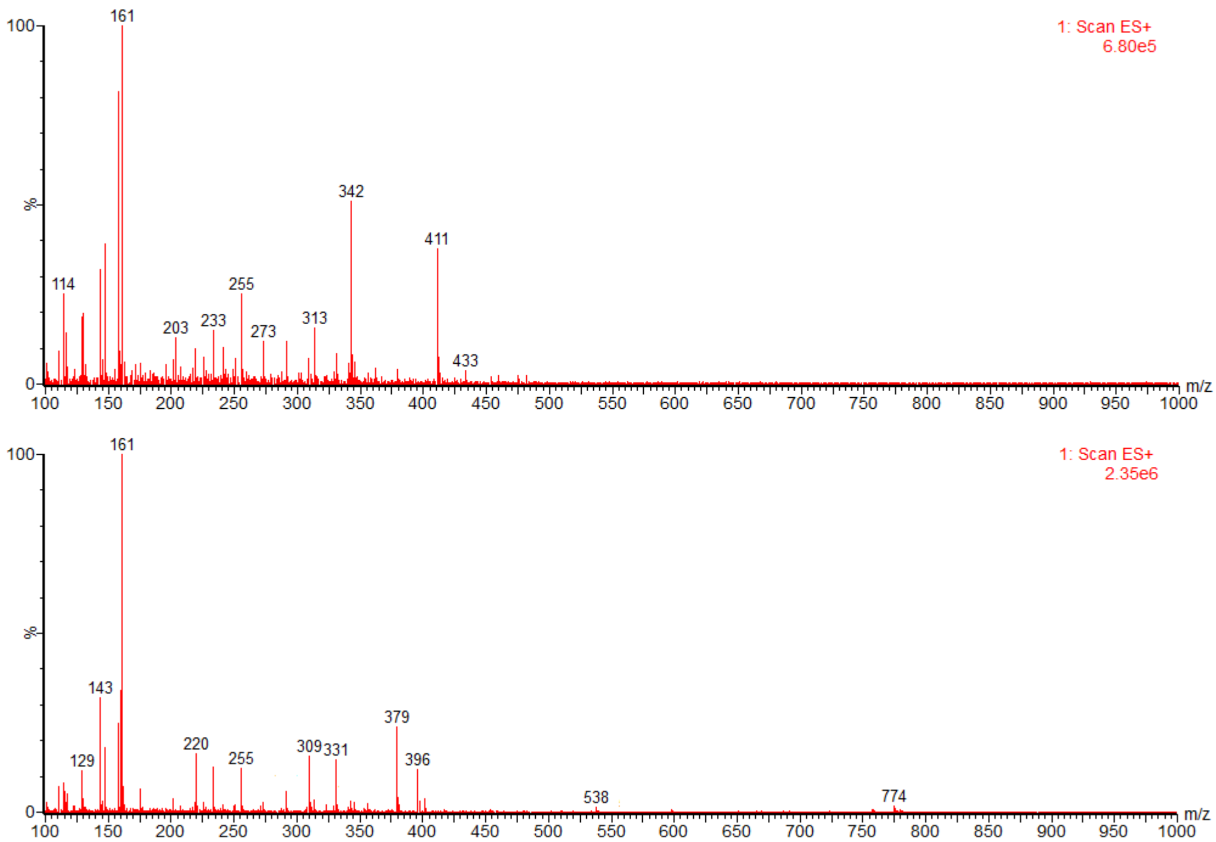

Figure 6s. Exemplary full scan mass spectra of carbenicillin methanolysis product (top) and carbenicillin (bottom) obtained upon HPLC/ESI-MS analysis.

The product ion spectra (CID-MS/MS spectra) were taken on a Waters/Micromass (Manchester, UK) Q-tof Premier mass spectrometer (software MassLynx V4.1, Manchester, UK). The sample solutions were infused into the ESI source by a syringe pump at a flow rate of 5 μL/min. The electrospray voltage was 2.7 kV and the cone voltage - 30 V. The source temperature was 80ºC and the desolvation temperature was 250ºC. Nitrogen was used as the cone gas and desolvating gas at the flow-rates of 50 and 400 L/h, respectively. Argon 5.0 was used as a collision gas at the flow-rate 0.2 ml/min in the T-wave collision cell. This flow rate resulted in the collision cell pressure 0.4 Pa. The most important parameter for CID-MS/MS experiments is collision energy (CE). The applied collision energy (laboratory frame), is indicated in each product ion spectrum shown.

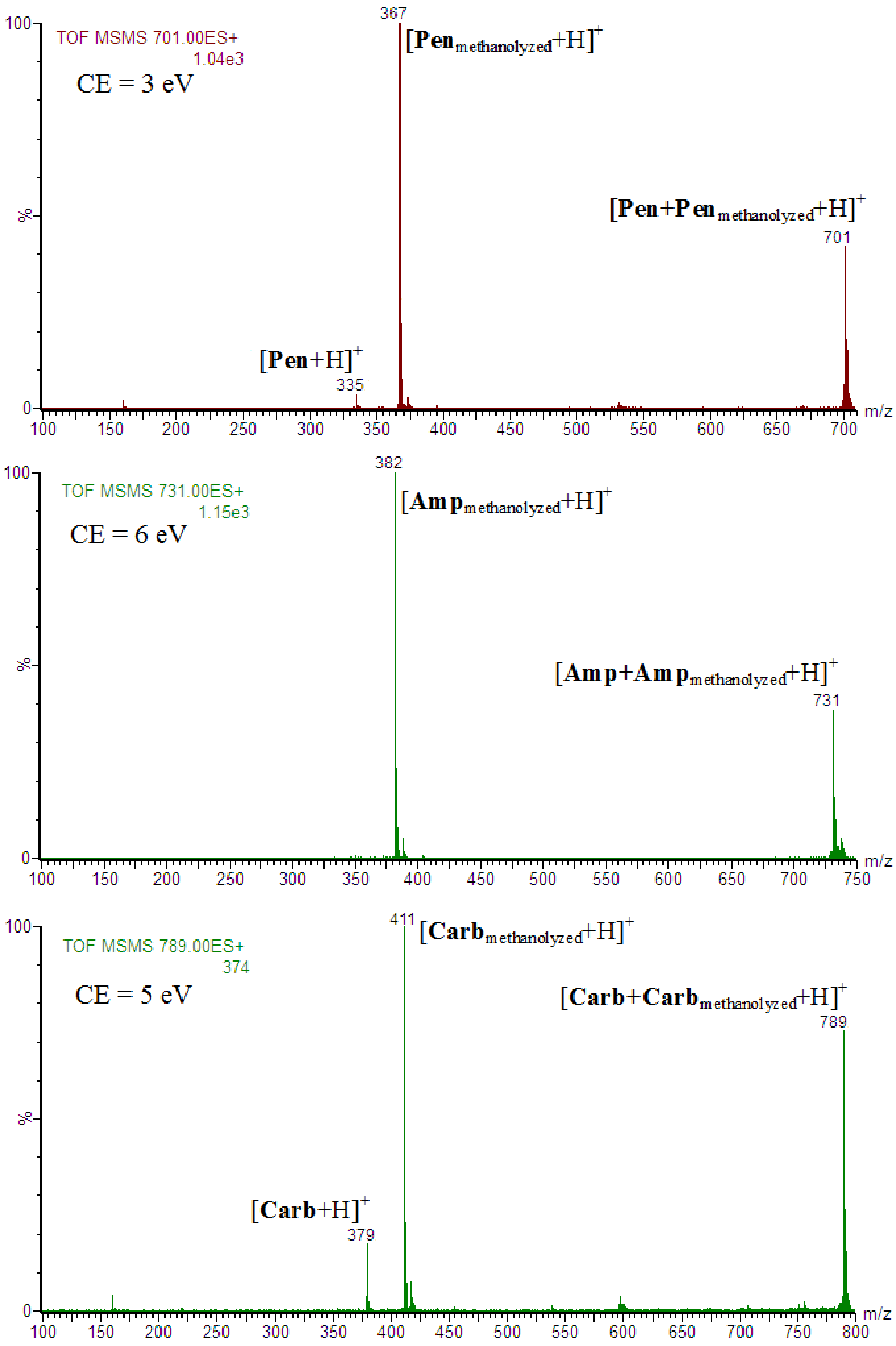

Figure 7s. The product ion spectra of adducts [**Pen**+**Pen**$_{methanolyzed}$+H]$^+$, [**Amp**+**Amp**$_{methanolyzed}$+H]$^+$ and [**Carb**+**Carb**$_{methanolyzed}$+H]$^+$. The adducts were characterized by low abundances, however, it was possible to obtain theirs product ion spectra.

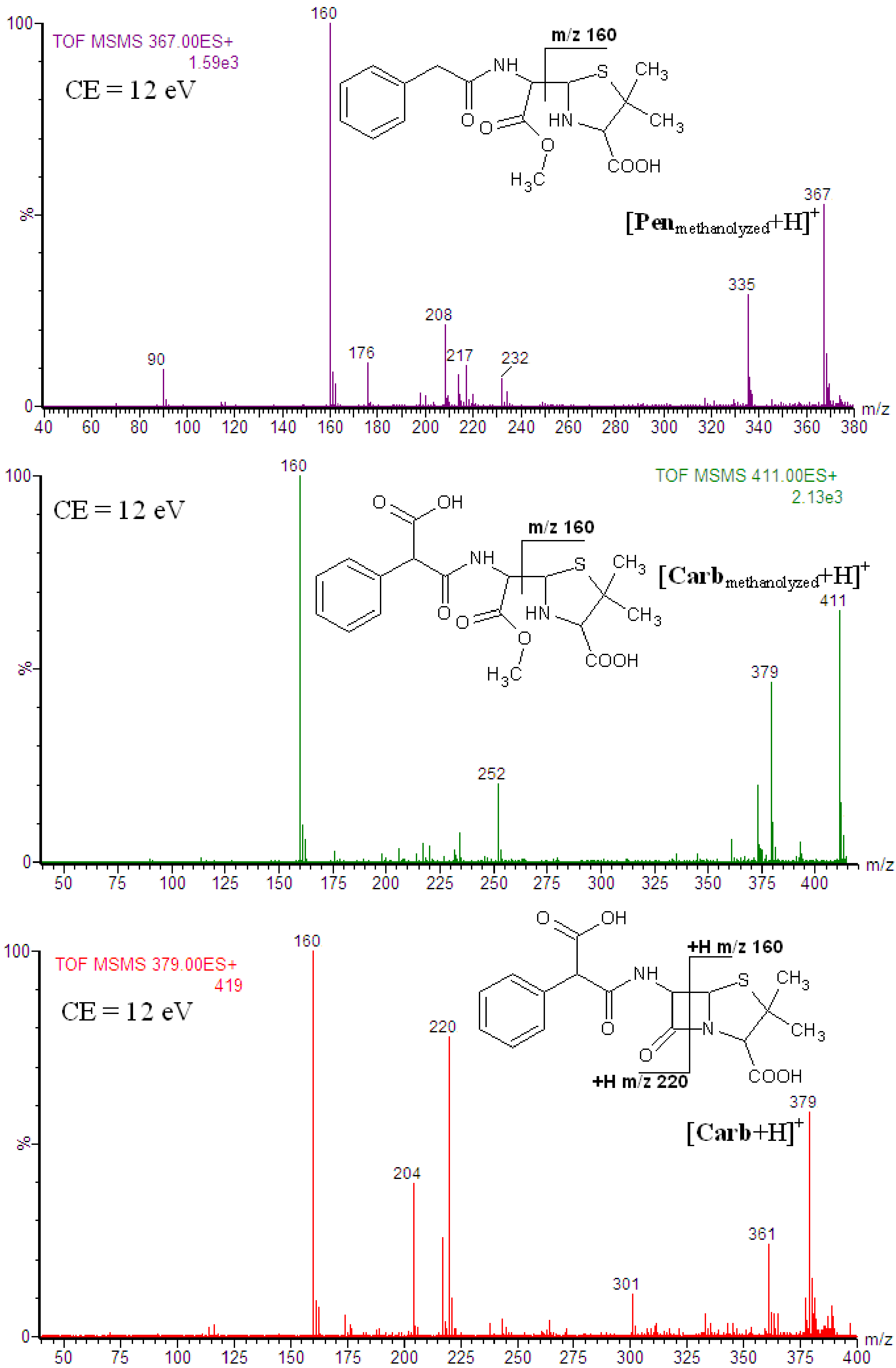

Figure 8s. Exemplary product ion spectra (CID MS/MS spectra) of [**M**+H]$^+$ ion of methanolyzed penicillin, methanolyzed carbenicillin, carbenicillin and formation of the most abundant fragment ions

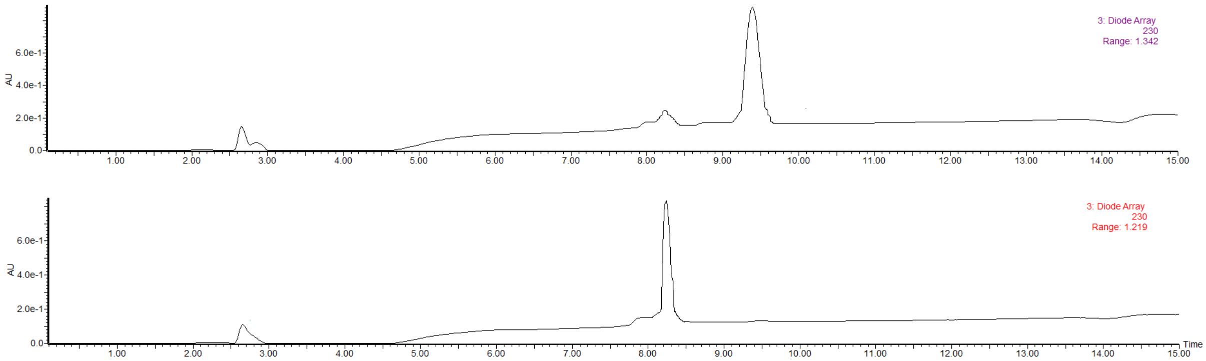

Figure 9s. HPLC/UV chromatograms obtained at 230 nm of methanolysed ampicillin (top) and ampicillin. Chromatographic conditions were identical as for HPLC/MS analysis and Waters 996 Photodiode Array Detector was used.

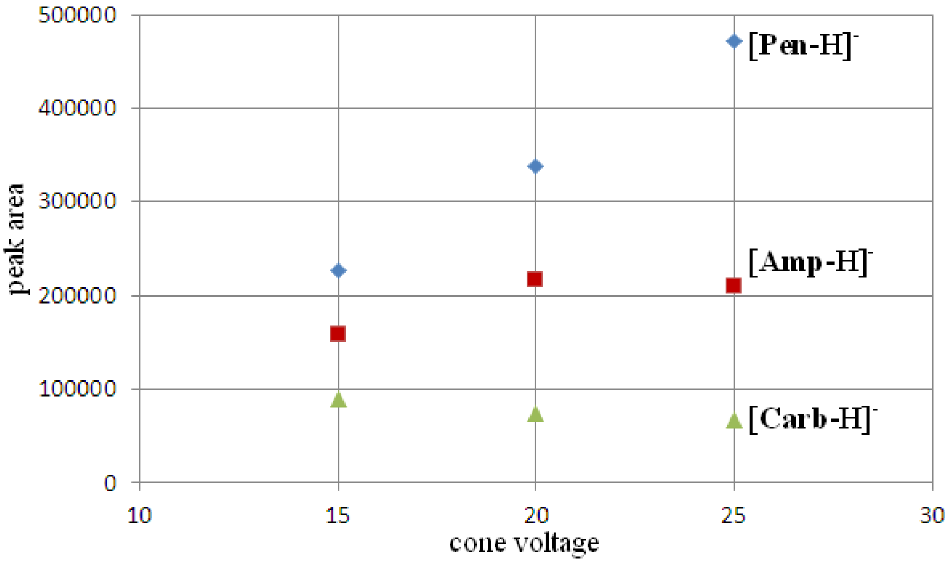

Figure 10s. The breakdown plots of the chromatographic peak areas of [**M**-H]$^-$ ions against cone voltage. It is a little unexpected that carbenicillin has the lowest ESI response in negative ion mode.

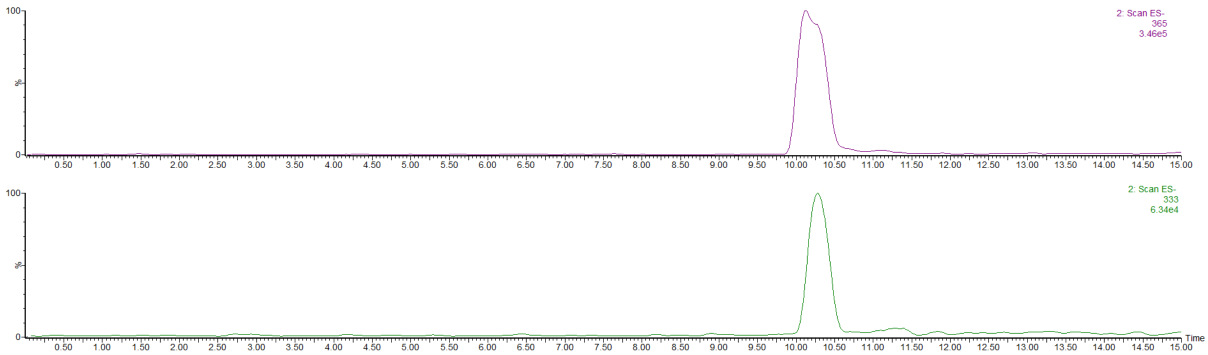

Figure 11s. Exemplary single ion chromatograms of [**Pen**$_{methanolyzed}$-H]$^{-}$ (m/z 365, top) and [**Pen**-H]$^{-}$ (m/z 333, bottom) obtained upon HPLC/ESI-MS analysis in negative ion mode.

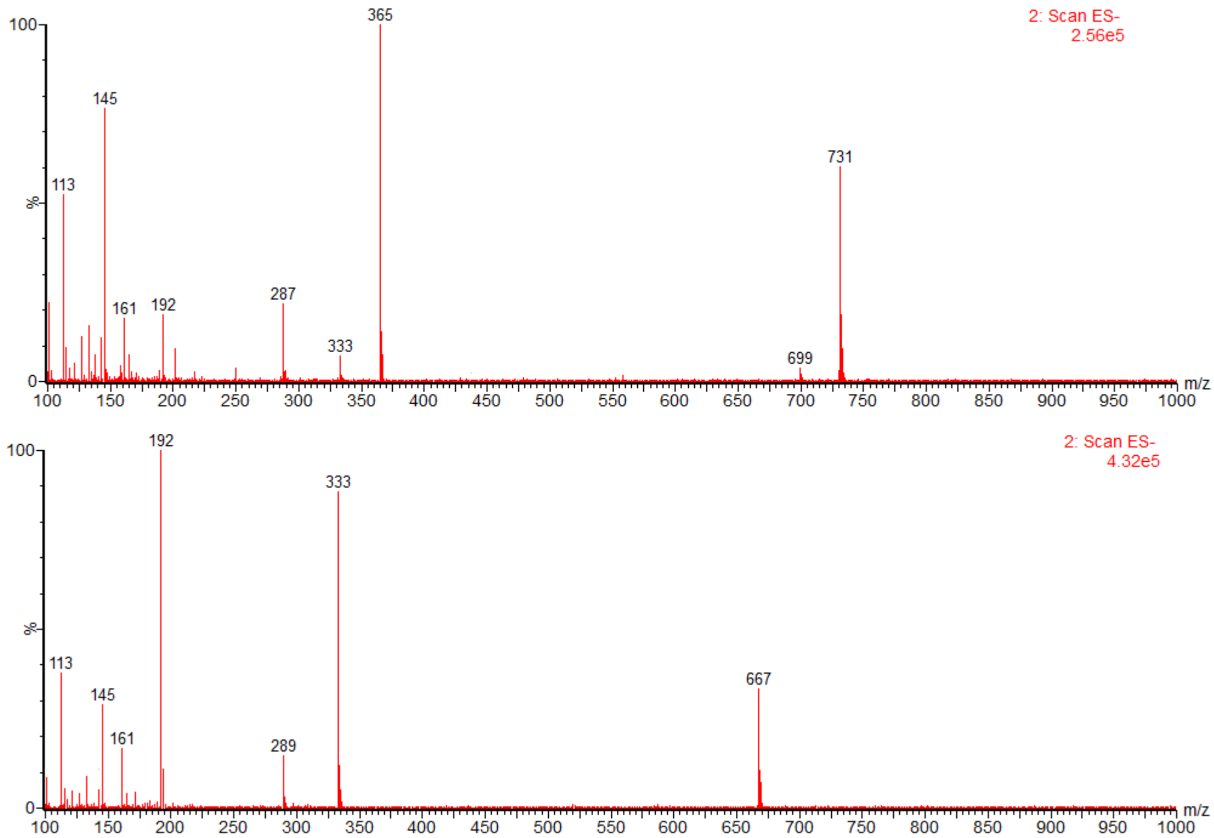

Figure 12s. Exemplary full scan mass spectra of penicillin methanolysis product (top) and penicillin (bottom) obtained upon HPLC/ESI-MS analysis in negative ion mode.

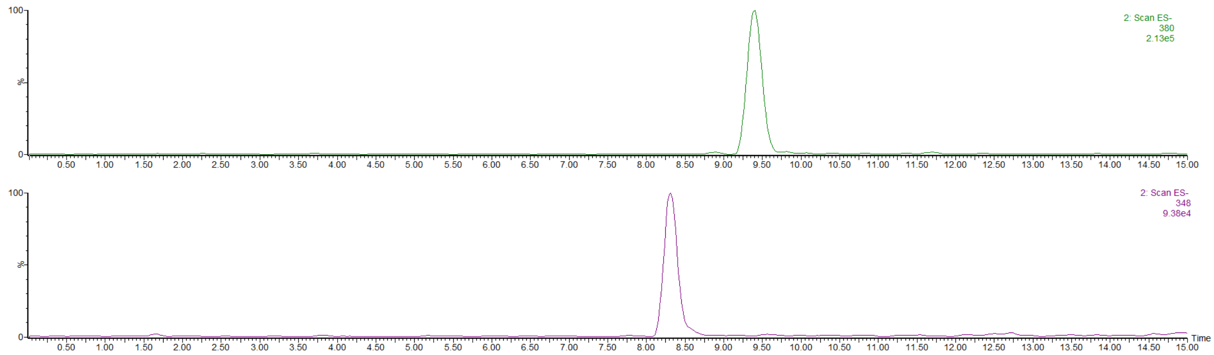

Figure 13s. Exemplary single ion chromatograms of [**Amp**$_{methanolyzed}$-H]$^-$ (m/z 380, top) and [**Amp**-H]$^-$ (m/z 348, bottom) obtained upon HPLC/ESI-MS analysis in negative ion mode.

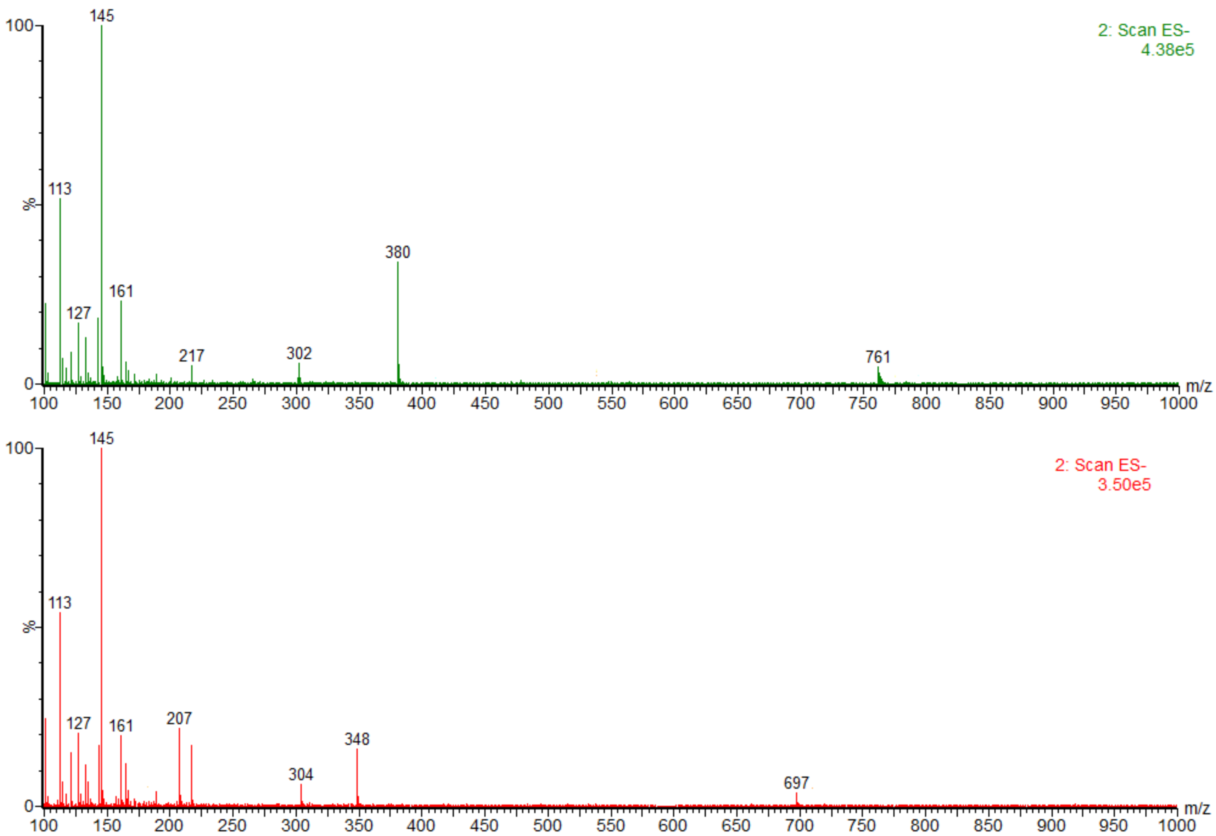

Figure 14s. Exemplary full scan mass spectra of ampicillin methanolysis product (top) and ampicillin (bottom) obtained upon HPLC/ESI-MS analysis in negative ion mode.

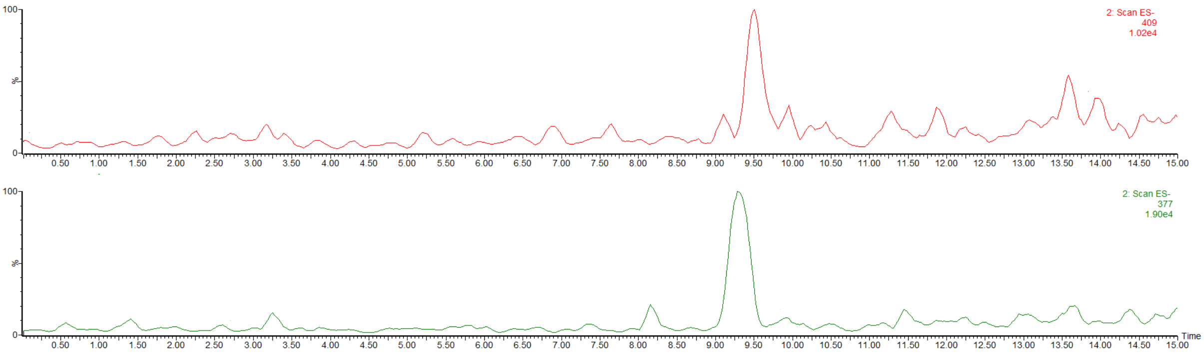

Figure 15s. Exemplary single ion chromatograms of [**Carb**$_{methanolyzed}$-H]$^-$ (m/z 409, top) and [**Carb**-H]$^-$ (m/z 377, bottom) obtained upon HPLC/ESI-MS analysis in negative ion mode.

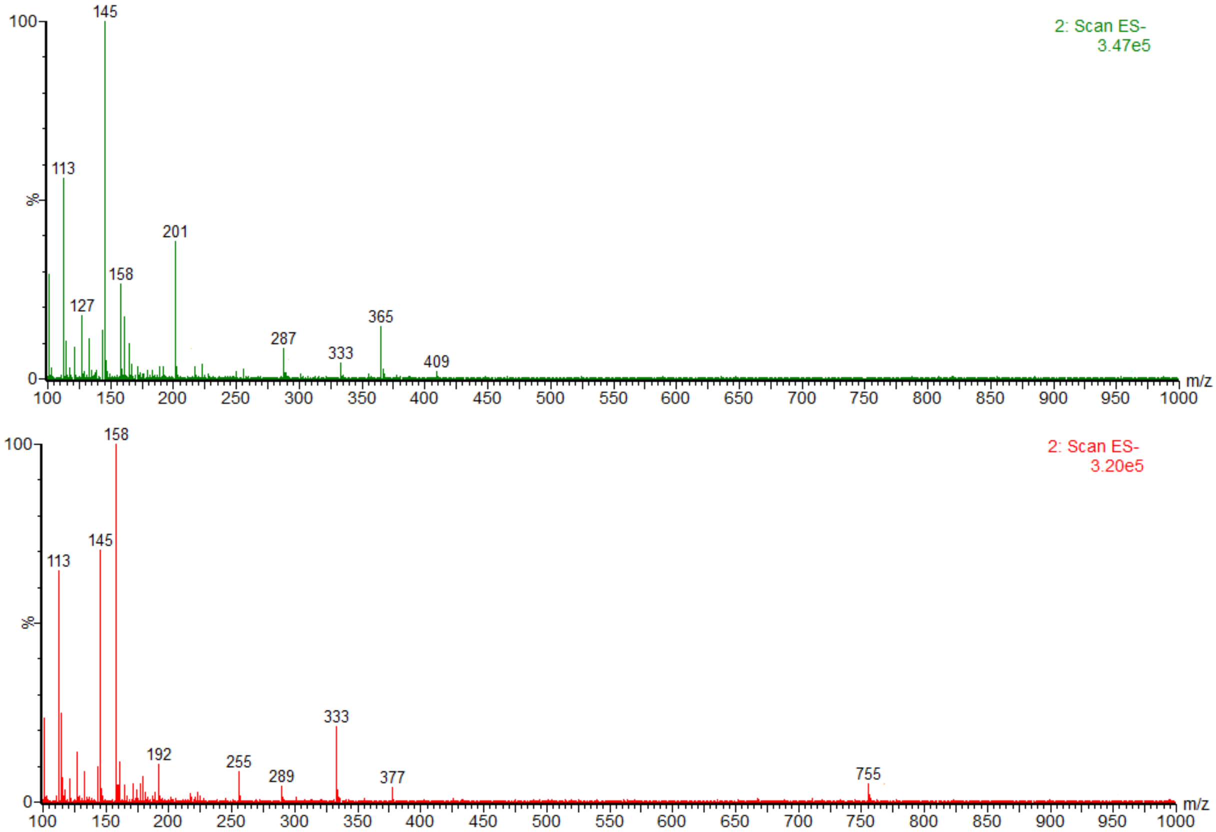

Figure 16s. Exemplary full scan mass spectra of carbenicillin methanolysis product (top) and carbenicillin (bottom) obtained upon HPLC/ESI-MS analysis in negative ion mode. The abundant fragment ions formed as a result of the loss of CO_2 molecules from [**M**-H]$^-$ ions are observed (m/z 365 for carbenicillin methanolysis product and m/z 333 for carbenicillin). Product ion spectra of [**M**-H]$^-$ ions confirm that the gas phase decomposition of [**Carb**$_{methanolyzed}$-H]$^-$ and [**Carb**-H]$^-$ occurs very easy (Figure 19s).

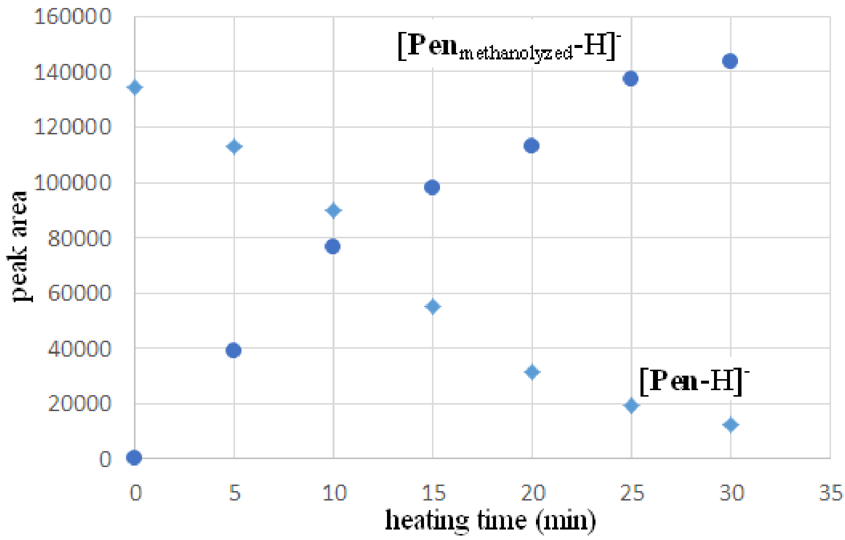

Figure 17s. The breakdown plots of the ESI response of ions [**Pen**-H]$^-$ (m/z 333) and [**Pen**$_{methanolyzed}$-H]$^-$ (m/z 365) against heating time. Methanolyzed penicillin almost identical ESI response as penicillin.

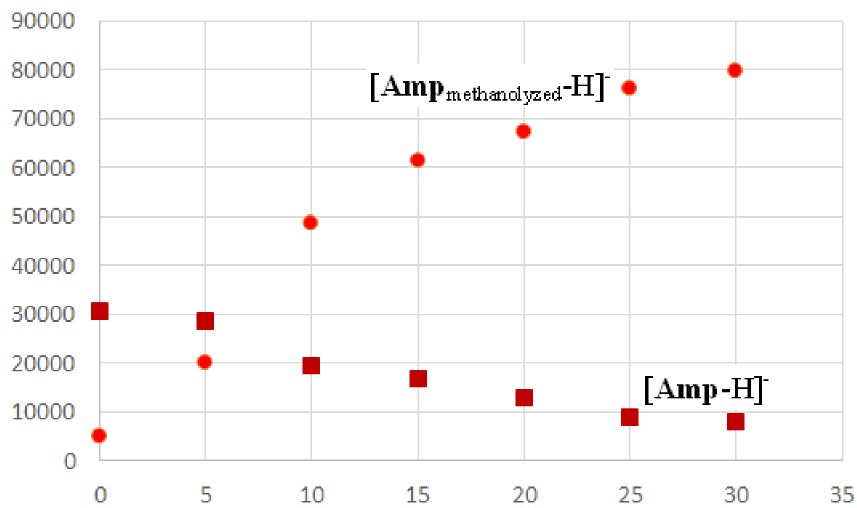

Figure 18s. The breakdown plots of the ESI response of ions [**Amp**-H]$^-$ (m/z 348) and [**Amp**$_{methanolyzed}$-H]$^-$ (m/z 38) against heating time. Methanolysed ampicillin has almost three times higher ESI response than ampicillin.

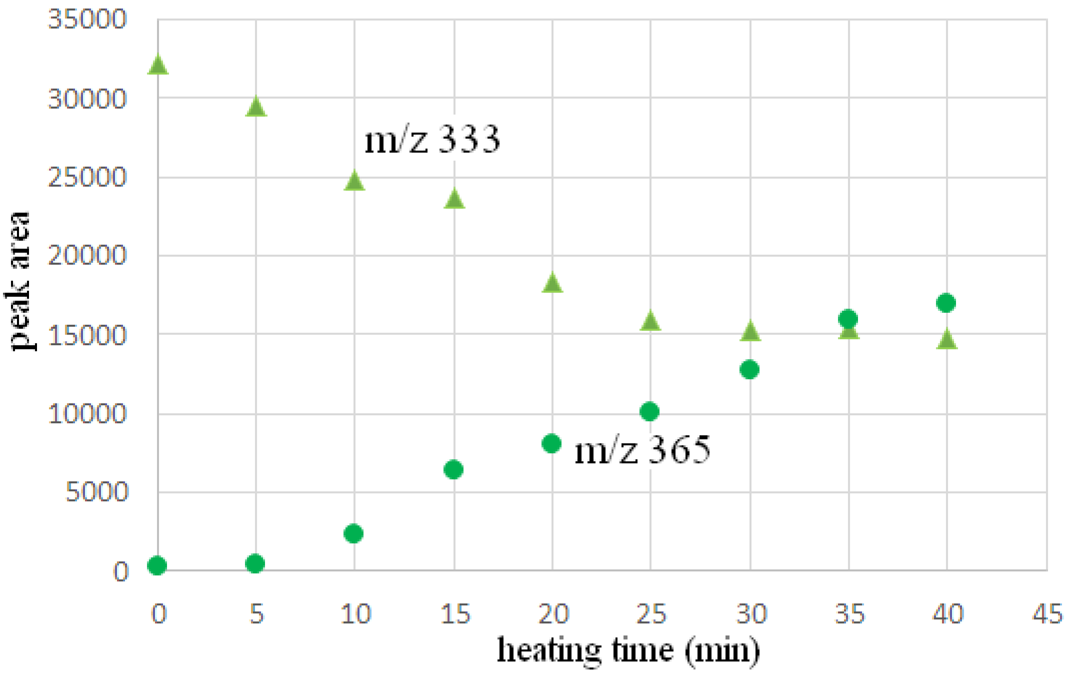

Figure 19s. The breakdown plots of the ESI response of ions [**Carb**-H-CO_2]$^-$ (m/z 333), [**Carb**$_{methanolyzed}$-H-CO_2] (m/z 365) against heating time (fragment ions formed as a result of the CO_2 loss are more abundant than deprotonated molecules (**Figure 14s**). Methanolyzed carbenicillin has lower ESI response than carbenicillin.

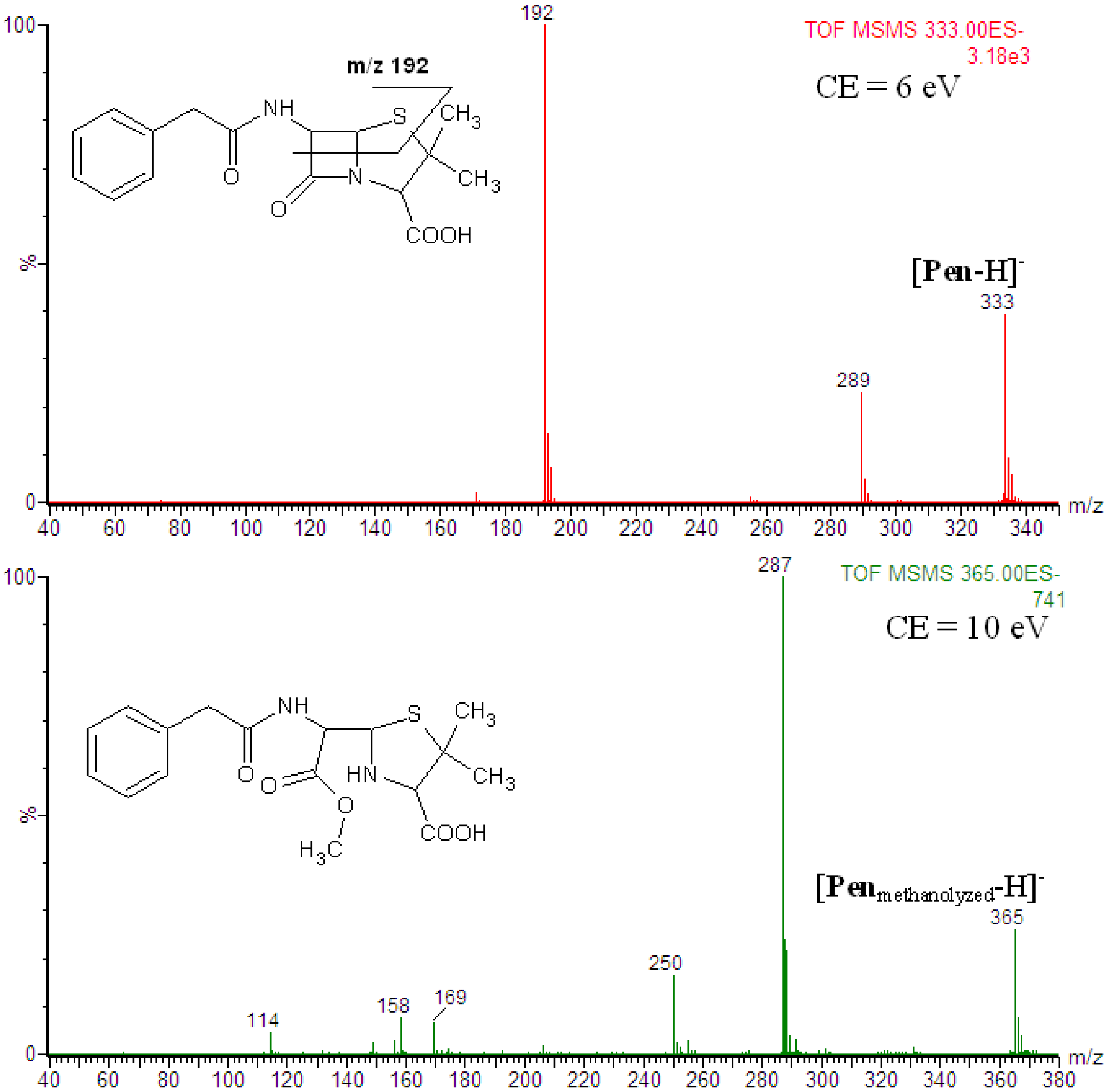

Figure 20s. Exemplary product ion spectra (CID MS/MS spectra) of [**M**-H]$^-$ ion of penicillin and methanolyzed penicillin.

Breaking of three bonds of bicyclic system (m/z 192) has been already observed for ampicillin and amoxicillin (Ghauch, et al. Environ. Pollut. 2009, 157, 1626-1635; Xi et al. Anal. Lett. 2012, 45, 1764-1776; Frański et al. Rapid Commun Mass Spectrom. 2014, 28, 713-722).

Fragment ion at m/z 287 is formed as a result of the loss of CO_2 and H_2S molecules form [**Pen**$_{\text{methanolyzed}}$-H]$^-$ ion, analogical processes have been observed for methanolyzed ampicillin and amoxicillin (Frański et al. Rapid Commun Mass Spectrom. 2014, 28, 713-722).

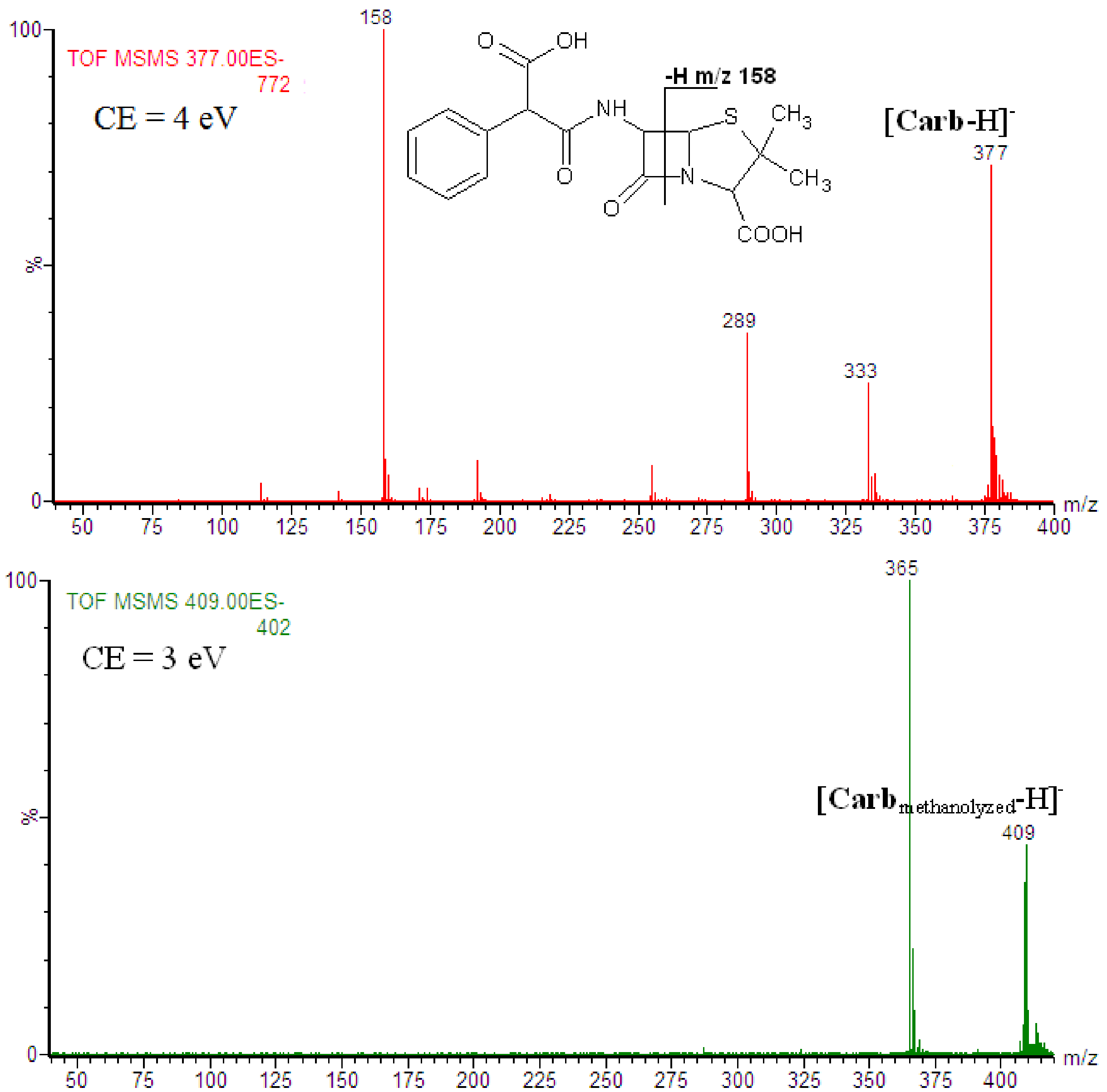

Figure 21s. Exemplary product ion spectra (CID MS/MS spectra) of [**M**-H]$^-$ ions of penicillin and methanolyzed penicillin. Abundant fragment ions are observed at low collision energy.