# Analytical method for the determination of amoxicillin and ciprofloxacin in the synthetic hospital wastewater

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## INTRODUCTION

Antibiotics are becoming increasingly problematic contaminants of water sources, especially in surface and underground water, which are located in the area of industrial and domestic communities. In the water resources they are mainly transited by discharges from pharmaceutical industries, hospitals and from municipal wastewater treatment plants (Yaghmaeian et al., 2014, Zuccato et al., 2010). The use of water, contaminated with antibiotics, can have many negative effects on people, including acute and chronic toxicity. Because antibiotics are designed to interfere with biological systems, their prolonged exposure can be harmful even in low concentrations (Yaghmaeian et al., 2014, Mojica and Aga, 2011, Zuccato et al., 2010). They can also have toxic effects on the cells, organs, organisms and populations. Increased use and exposure to antibiotics over the last few decades, increased bacterial resistance against them. In this study, we focused on two most commonly used antibiotics in Slovenia, ciprofloxacin and amoxicillin, whose concentration in effluents ranged between 50 and 600 ng/L, in raw hospital waste water up to 125 µg/L (De Witte et al., 2009).
The purpose and aim of the study is to develop a sensitive, reproducible and useful method for the determination of selected antibiotics in hospital wastewater.

## METHODS AND MATERIALS

The samples that we used were synthetically prepared waste water, in which we added an appropriate volume of the mixture of the standard solution of ciprofloxacin and amoxicillin, so that the concentration in our samples was 0,8 mg/L. The measurements were carried out on a liquid chromatograph (Agilent Technologies 1260 Infinity), coupled with a mass spectrophotometer (MS/MS, API 2000). Analytes were identified and quantified, by using a linear calibration curve, in the range of 50 to 1000 ng/mL. We used the isotopic dilution method. For the separation and quantification of antibiotics and their isotopic compounds we used a Phenomenex Synergen Fusion-RP analytical column of 100 A (50 x 2.0 mm, 2.5µm). The LC and MS/MS conditions are shown in the tables below:

| | |
|---|---|
| **Mobile phase A** | 0,1 % formic acid in a solvent mixture of water and acetonitrile in a ratio of 95:5 |
| **Mobile phase B** | 0,1 % formic acid in a solvent mixture of methanol and acetonitrile in a ratio of 50:50 |
| **Flow** | 300 µl/min |
| **Column temperature** | 40 °C |
| **Injection volume** | 50 µl |

| Time (min) | A (%) | B (%) |
|---|---|---|
| **0** | 100 | 0 |
| **2** | 100 | 0 |
| **7** | 0 | 100 |
| **7.10** | 100 | 0 |
| **17** | 100 | 0 |

Table 1 and 2: LC gradient elution program of amoxicillin and ciprofloxacin

| Compound | Q1 mass (Da) | Q3 mass (Da) | CE (V) | Q1 mass (Da) | Q3 mass (Da) | CE (V) |
|---|---|---|---|---|---|---|
| **Ciprofloxacin** | 332.16 | 231.2 | 51.00 | 332.16 | 288.2 | 23.00 |
| **Amoxicillin** | 366.171 | 114.1 | 25.00 | 366.171 | 208.2 | 19.00 |
| **Ciprofloxacin-D18** | 340.228 | 235.200 | 53.00 | 340.228 | 296.400 | 23.00 |
| **Amoxicilin 13C6** | 372.163 | 114.100 | 27.00 | 372.163 | 214.300 | 19.00 |

| | |
|---|---|
| **Scan mode** | MRM |
| **Polarity** | positive |

Table 3 and 4: MS/MS conditions used for the detection of amoxicillin and ciprofloxacin

## RESULTS

In the initial phase of the development of analytical method, we evaluated a little higher concentrations of antibiotics, which ranged between 50 and 1000 ng/mL. This way, we could avoid the sample preparation. The method for determining the content of selected antibiotics, by liquid chromatography coupled with tandem mass spectrometry, has proven to be very selective and highly sensitive method. The instrument response of selected antibiotics was linear (r2≥0,99). For the evaluation we used isotope dilution method, where the highest ion response was used for the quantitative analyse and the responses of other ions of the same analyte, confirm its identity.

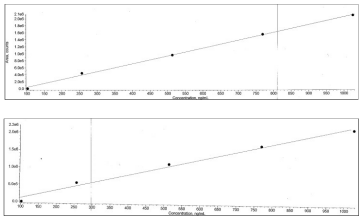

Figure 1 and 2: Linear response of amoxicillin and ciprofloxacin

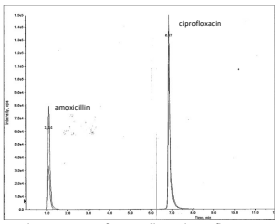


Figure 3: Chromatogram of amoxicillin and ciprofloxacin

## CONCLUSION

Large production of antibiotics in the world, where the annual production is 100,000 to 200,000 tons, is one of the main reasons for the gradual increase of pharmaceutical contaminants in the water. Antibiotics are often difficult to biodegrade, which affects their efficient removal by conventional water treatment plants. Although the exact impact of this kind of pollution on living beings, is not yet entirely clear, it is a serious problem indirectly linked to the spread of bacterial resistance to antibiotics. Therefore, it is necessary to remove the antibiotic completely before they reach natural waters.

## LITERATURE

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