Quantification of veterinary antibiotics of three classes in soil extracts and natural water using spectral methods

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Introduction

The uncontrolled spread of antibiotics in wastewater and natural environments currently poses a serious environmental threat in all countries. This is primarily due to the accumulation of genes of resistance to pharmacological drugs in bacteria, as well as the disruption of the stable functioning of all trophic levels of ecosystems. In such industries as agriculture, veterinary medicine and fish farming, pharmaceuticals are widely used not only as therapeutic and prophylactic agents, but also to stimulate growth. Therefore, the development of rapid methods for detecting antibiotics in natural environments is extremely relevant.

Objective and tasks

The work studied the spectral characteristics of three veterinary antibiotics (tylosin, tetracycline, ciprofloxacin) of various concentrations in the presence of organic substances of natural origin or by-products of animal husbandry (dissolved organic matter of natural water, aqueous extracts of soils and organic fertilizers).

Materials and methods

Ciprofloxacin (ready-to-use 2 mg/mL solution) was obtained from Mosfarm (Russia). Tylosin (200 mg/mL solution) was acquired from Nita-farm (Russia). Tetracycline hydrochloride was purchased from Sigma-Aldrich.

UV-Vis absorption spectra were measured in quartz cuvettes with a 1 cm path length using a Solar PB2201 spectrophotometer over the wavelength range of 200–500 nm, and fluorescence spectra were registered using a Solar CM 2203 luminescence spectrometer. Emission spectra were corrected for the inner-filter effect using absorbance values at excitation and emission wavelengths.

Results

Absorption spectra

All three antibiotics demonstrated absorption bands in the UV region, which were separated by mathematical processing from the broad background of absorption of natural organic matter of water, soil or organic fertilizers, monotonically decreasing with rising wavelength. The concentration of the antibiotic was determined by the area of the absorption band or optical density at a fixed wavelength using calibration dependencies. In this way, it is possible to determine concentrations of tylosin up to 100 mg/l, and for tetracycline and ciprofloxacin up to 60 mg/l. At higher concentrations it is necessary to dilute the samples or use the absorption at longer wavelengths.

The minimum concentrations of antibiotic that can be detected using spectrophotometry are determined by the instrumental error of the spectrophotometer. For a cuvette with an optical path length of 1 cm, we estimate the lower limit of concentration measurement as 1 mg/L for tylosin and 2 mg/L for tetracycline and ciprofloxacin in water.

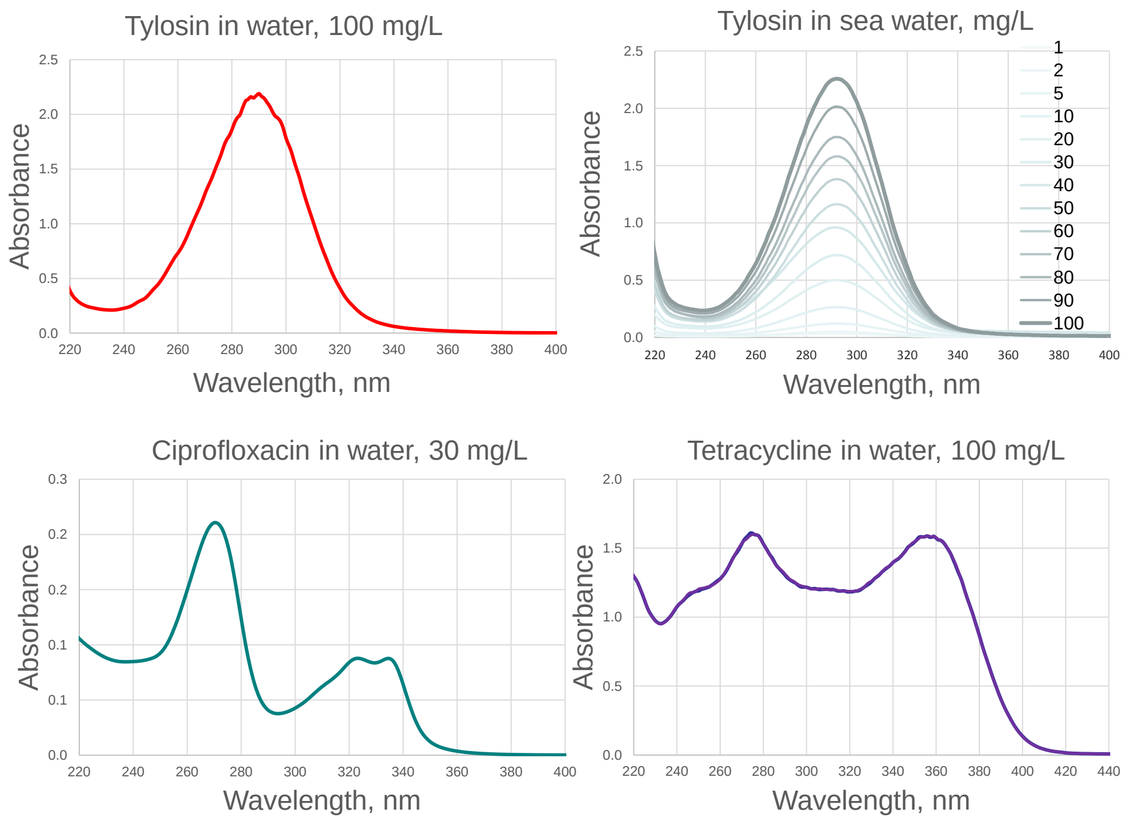

Figure 1. Absorption spectra of solutions of tylosin in distilled water and seawater, ciprofloxacin and tetracycline in distilled water.

Antibiotics and organic matter of the environment

However, when measuring the antibiotic concentration by spectrophotometry in natural water, soil or in the presence of organic fertilizer, it is necessary to take into account the absorption of light by the dissolved organic matter (DOM) of the water/soil/fertilizer. It is known that the absorption spectrum of natural humic substances (chromophoric fraction of DOM in natural water or soil) decreases monotonically with increasing wavelength. Sometimes, a small local maximum or inflection in the wavelength region of 260-270 nm can be seen in the absorption spectra of natural DOM in the UV range, associated with the presence of phenolic or protein compounds. However, there are no clearly defined absorption bands in the UV range for natural humic substances. A similar picture was observed for the aqueous extract of organic fertilizer based on manure. The absorption bands of antibiotics when they are added to the manure filtrate are less intense compared to the absorption of the OM of the organic fertilizer.

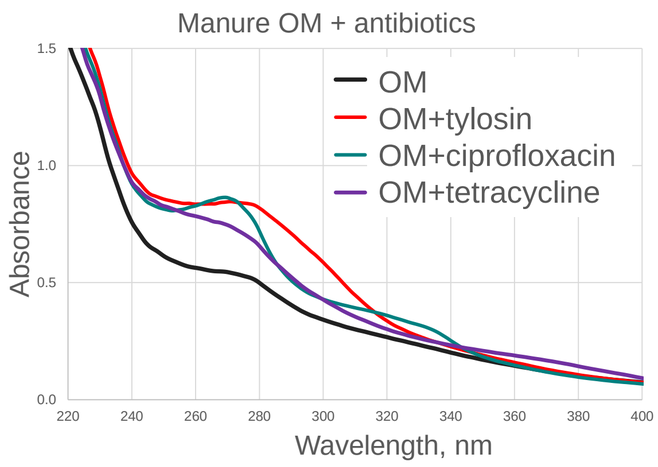

Figure 2. Absorption spectra of OM from manure-based organic fertilizer in water with added antibiotics.

To measure the concentration of antibiotics, the areas under the UV absorption bands of antibiotics were taken. The dependences of the absorption band area on the concentration for the corresponding preparation in water were used as a calibration dependence. This method allows measuring the concentrations of antibiotics in natural water, soil extracts or organic fertilizer solutions up to approximately antibiotic concentrations of 5-10 mg/L. For lower concentrations of antibiotics, more sensitive fluorescent methods should be used.

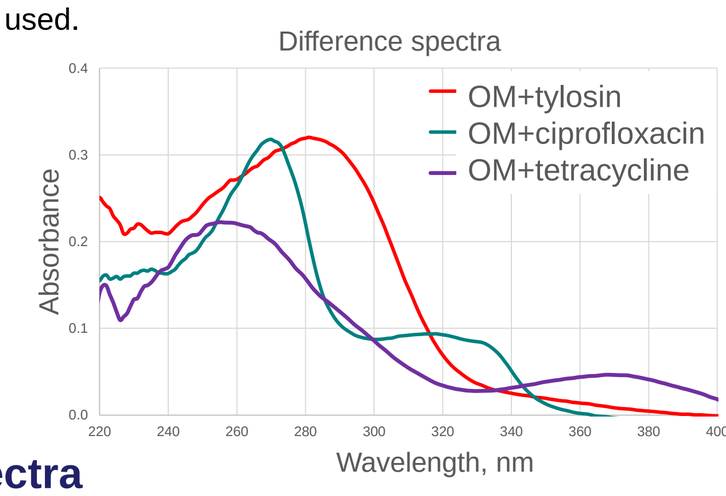

Figure 3. Difference absorption spectra of antibiotics in manure filtrate after subtracting the absorption spectrum of the manure filtrate.

Fluorescence spectra

Fluorescence spectroscopy was used to monitor concentrations of ciprofloxacin below 5 mg/L. The emission band of ciprofloxacin partially overlaps with the fluorescence of organic matter in water and soil, so to isolate the contribution of this antibiotic to the spectra, synchronous fluorescence spectra were measured with a constant difference in excitation and recording wavelength. In this case, the antibiotic concentration was determined by the area of the synchronous fluorescence spectrum band in the UV range 270-370 nm after subtracting a similar spectrum measured for a sample without antibiotic.

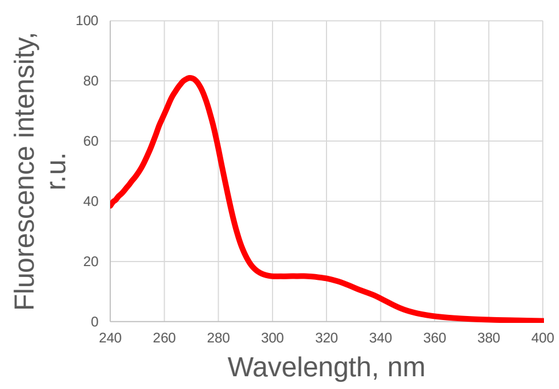

Figure 4. Synchronous fluorescence spectrum of ciprofloxacin solution of 5 mg/L concentration, the offset between the excitation and recording wavelength is 160 nm.

Tylosin in aqueous solutions did not exhibit fluorescence emission, and the fluorescence band of tetracycline overlaps with the fluorescence bands of the components of soil organic matter or organic fertilizer. That makes difficult their fluorescent diagnostics.

Conclusions

The research shows that the veterinary antibiotics tylosin, tetracycline, ciprofloxacin can be quantitatively measured by absorption spectroscopy in various aqueous media, including those containing soil organic matter or organic fertilizers. Fluorescence spectroscopy, although a more sensitive method than absorption spectroscopy, has its limitations: tylosin does not exhibit fluorescent properties in water, and the luminescence band of tetracycline overlaps with the fluorescence bands of soil organic matter components or organic fertilizers.

For one of the drugs, ciprofloxacin, it was possible to develop a method for determining low concentrations of the antibiotic (less than 5 mg/L) in aqueous media with a significant content of organic matter by measuring synchronous fluorescence spectra with a constant shift between the excitation wavelength and the emission wavelength. We consider the possibility of measuring antibiotic concentrations in organic fertilizer extracts or in the presence of soil organic matter as a promising methodological direction in improving the system of standardizing the safe content of chemical pollutants from the group of non-traditional environmental toxicants – pharmaceutical antibiotics.

Acknowledgments

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