Chemia Naissensis, Vol 6, Issue 1, RESEARCH ARTICLE, 49-63 **DOI: 10.46793/ChemN6.1.49N**

Dispersive solid phase extraction for antibiotics analysis

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ABSTRACT

Antibiotics are widely used to prevent diseases and promote growth in food - producing animals. Their usage may result in the presence of antibiotics in food and environmental samples. Antibiotics analysis in complex samples, such as food and environment samples, require sample pretreatment.

Application of activated carbon, C18 and florisil and the influence of the amount of applied sorbents on their effectiveness in dispersive solid phase extraction (dSPE) for chloramphenicol and tetracycline analysis was examined. Activated carbon showed the lowest efficiency in the extraction of antibiotics (29% when analyzing samples containing chloramphenicol when 0.05 g of sorbent was added). When analyzing samples containing chloramphenicol, florisil showed an equal efficiency for all three sorbent amounts (92%), so extraction efficiency when using florisil does not depend on the mass of applied sorbent. Octadecyl silica (C18) shows high efficiency when analyzing chloramphenicol and tetracycline (96% in samples containing chloramphenicol, and 102% in samples containing tetracycline), so it can be applied in chloramphenicol and tetracycline analysis.

Keywords: *Antibiotic, sorbents, dSPE*

Introduction

Antibiotics are specific products of the metabolism of microorganisms that have a high physiological activity towards other groups of microorganisms (bacteria, molds, protozoa), preventing their growth and destroying them. In addition to natural antibiotics, obtained by microbial biosynthesis, there are also semi-synthetic and synthetic antibiotics (Spahija., 2020).

Apart from their origin, antibiotics also differ in their chemical composition and action on organisms. These drugs belong to a group of useful antimicrobial compounds that are widely used in human and veterinary medicine. Veterinary drugs, especially antibiotics are among the most important associations related to fodder production. Approximately 80% of animals used in food production are treated with antibiotics and other veterinary drugs in a certain period, or throughout life (Pavlov et al., 2008). The main use of antibiotics in animals is in the treatment and prevention of diseases such as mastitis, arthritis, respiratory infections, gastrointestinal and others bacterial infections (Darwish et al., 2013). Veterinary antibiotics were originally used in animals to disease treat and prevention, but today they are gradually added to food for reproduction control cycles and improvement of animal traits and as growth promoters, which goes far beyond their use as therapeutics for animals.

There is often excessive use of antibiotics and their misuse, which results in the appearance of increasing resistance to microorganisms, but also environmental pollution. Most antibiotics are incompletely metabolized after consumption and are excreted in unchanged form into the environment. In the environment they can accumulate and pollute the environment, but also enter food chain. Researchers have shown that antibiotics produced by humans most often reach the environment from the production facilities of the pharmaceutical industry, through excretion after use or discarding unused antibiotics. In a few cases, concentrations of antibiotic residues exceed 1

mg L^{-1} in treated industrial waters, which is much higher than the concentrations that are regularly detected as a consequence of antibiotic excretion (Larsson, 2014).

The degradation of drugs in the environment depends on the chemical properties of the active substance (Robinson et al., 2007). Many drugs are lipophilic and easily pass through cell membrane and bioaccumulate in aquatic animals. In the aquatic environment they are transformed by abiotic and biotic processes. Some antibiotics, such as penicillin's, are easily degraded, while others are significantly more persistent, for example tetracyclines and fluoroquinolones, which allows them to retain and spread in the environment and accumulate to higher concentrations (Larsson, 2014; Li et al., 2008a, Li et al., 2008b).

Residues of these drugs can lead to various toxic effects such as allergy, immunopathological effects, nephropathy, hepatotoxicity, mutagenicity, carcinogenicity, effects to reproductive health and even anaphylactic shock in humans (Darwish et al., 2013; Nisha, 2008). Antibiotics used in veterinary medicine can come into contact with soil, and by washing the soil, they reach the underground water (Heberer, 2002).

Analysis of antibiotics in complex samples requires sample pretreatment and is a challenge for researchers, because of the low concentrations of antibiotics in the samples, but also similar analytical signal given by the individual components. One of the newer techniques, which is more often used in sample preparation is QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) technique. It consists of extracting the analyte with a suitable solvent and purification of the obtained extract by dispersed solid phase extraction (dSPE).

Dispersive solid phase extraction is an improved version of solid phase extraction due to the direct addition of the sorbent to the sample solution, thereby increasing the surface of the contacting phases. dSPE consists of adding a solid sorbent, usually silica or polymer based directly to the

sample solution, whereby the dispersion process increases the contact area between the sorbent and the solvent. Sorbents used for the determination of antibiotic residues in samples are solid substances that have undergone chemical modification by the addition of some chemical compounds, which increases their selectivity, longer contact with the analyte and minimal matrix interference during analysis (Xiong et al., 2015; Barrado and Avila, 2019). The selection of sorbents for dSPE is a challenge for researchers, due to the large number of sorbents that their selectivity towards analyte and co-extracted impurities.

In this paper, the possibility of using florisil, C18 and activated carbon for analysis of chloramphenicol and tetracycline in samples was examined. After appropriate treatment, efficiency was determined, in order to point out which sorbent can be used in dSPE. The samples were analyzed by high-resolution liquid chromatography, which achieves efficient separation of analyzed antibiotics on the HPLC column.

Experimental

Chemicals and reagents

Chloramphenicol (HPLC grade), tetracycline (HPLC grade), methanol (HPLC grade), acetonitrile (HPLC grade) were purchased by Sigma Aldrich, Germany. Boric acid (HPLC grade), hydrochloric acid, distilled water, activated carbon for the HPLC were purchased by Merck, Darmstadt, Germany, whereas C18 (octadecylsilane) was purchased by United Chemical Technologies UCT, Horsham, USA.

Preparation of the solution

Solutions of chloramphenicol and tetracycline in methanol are prepared in concentration of 5 mg mL^{-1}.

The mobile phase for HPLC analysis is prepared from boric acid aqueous solution (20 g L^{-1}) and acetonitrile (60 + 45 mL). The pH of the solution was adjusted up to 3 with 2 mol L^{-1} HCl solution.

Samples pretreatment for HPLC analysis

C18, florisil and activated carbon (0.05 g; 0.1 g and 0.2 g) are weighted into QuEChERS tube.

1 mL of the antibiotic solution is transferred to each of them with and mixtures were shaken for 1 minute and centrifuged at 8000 rpm for 10 minutes (Cvetković, 2016). The supernatant was filtered through a membrane filter (0.45 μm) directly into the vial for HPLC analysis.

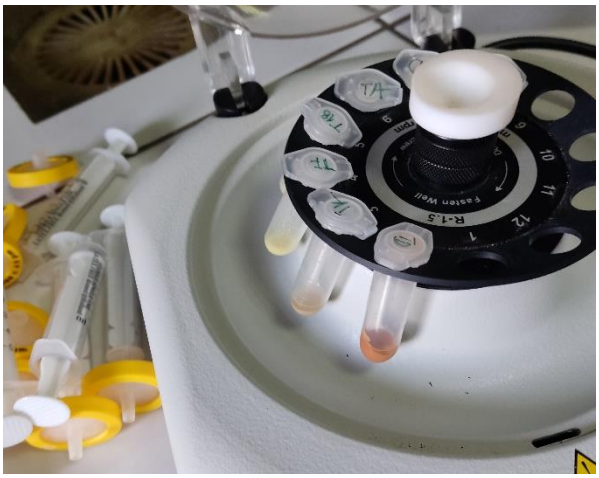

Figure 1. Sample pretreatment

HPLC analysis

The analysis was performed using the Agilent 1200 Technologies HPLC apparatus which is equipped with a vacuum degasser, a quaternary pump, a sample injector, a column and UV detector, controlled by Agilent software.

Separation of sample components is achieved on a Zorbax Eclipse plus C18 (150 x 4.6 mm, 5 μm) analytical column, at a temperature set at 35 °C, with gradient elution of the mobile phase.

Solvents are mixed in a gradient starts with 20% acetonitrile, which is held for 2 minutes, and then increases linearly to 50% over 12 minutes. After that, the initial conditions were held for 5 minutes to flush the system before the next sample injection. The analysis is performed at a mobile phase

flow rate of 1 mL min^{-1}. The absorption wavelength was measured at 220 nm. (Pietron, 2014) Antibiotic concentration was calculated using calibration curve (Table 1), and after that was calculated:

$$\text{Efficiency (\%)} = \frac{C_A}{C_s} \times 100\%$$

C_A - antibiotic concentration after treatment

Cs – antibiotic concentration before treatment

Table 1. Identification and quantification parameters

Antibiotic	Rt (min)	Calibration curve equation	r^2
Chloramphenicol	3,83	y=1,635*x+0,0002	0,99
Tetracycline	1,913	y=1,766*x +0,0001	0,99

Results and Discussion

Choosing the proper sorbent for dSPE is of crucial importance for the entire analytical procedure. In this paper, the possibility of using C18, florisil and activated carbon and appropriate amount of sorbent for the analysis of chloramphenicol and tetracycline was examined.

Efficiency was calculated by comparing the concentrations obtained after sample pretreatment by dSPE and initial concentrations of antibiotics.

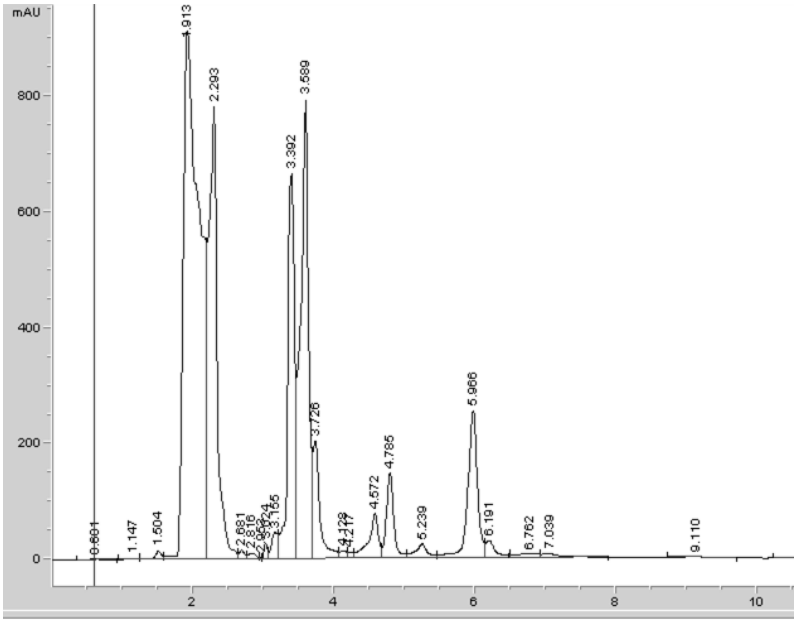

Figure 2. Chromatogram of tetracycline

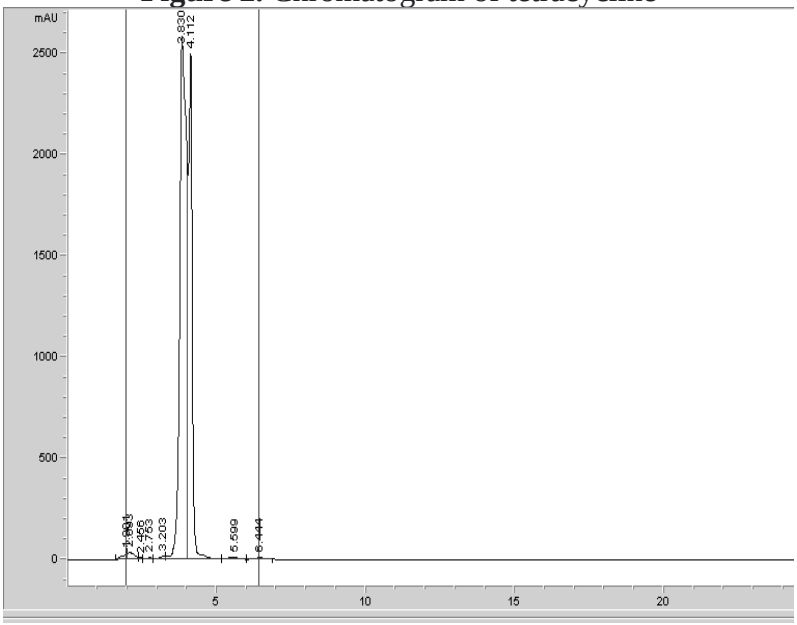

Figure 3. Chloramphenicol

C18 is one of the most commonly used sorbents in solid phase extraction and has been used so far in the analysis of chloramphenicol by solid phase extraction (Śniegocki et al., 2017). C18 shows high efficiency when analyzing chloramphenicol and tetracycline. Efficiency values obtained for chloramphenicol analysis, with the increasing amount of sorbent were: 92, 92 and 96%, respectively. These results fall within the acceptable range of extraction efficiency prescribed by good ISO (Thompson et al., 2002).

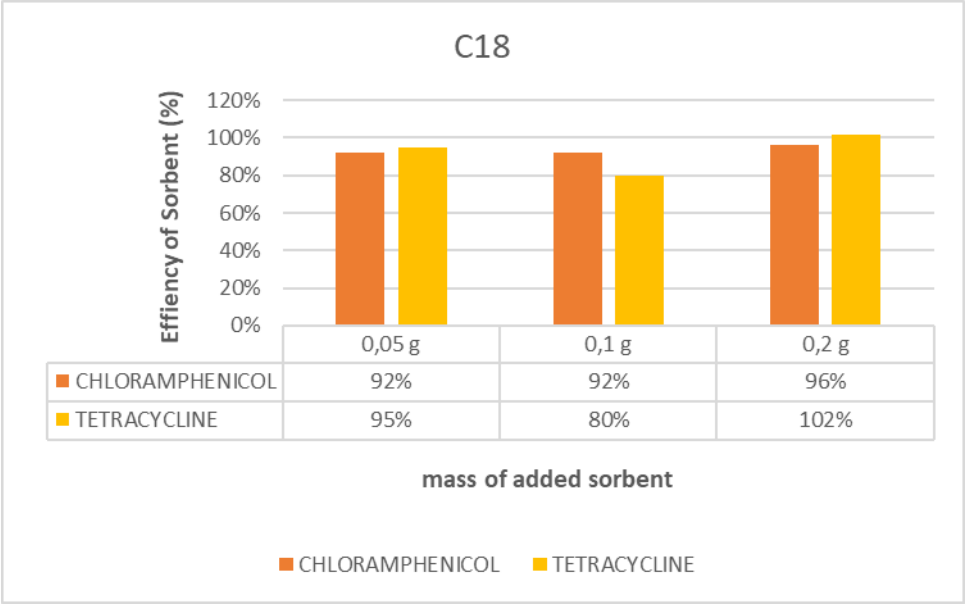

Figure 4. The influence of the mass of C18 as a sorbent on the extraction efficiency of antibiotics

When analyzing tetracycline, there are some deviations in the results. At the amount of 0.05 g of sorbent, the efficiency is 95%, at the amount of 0.1 g, it is 80%, while 0.2 g of the sorbent gives efficiency of 102%. Based on these results, it can be seen that there is a difference in efficiency when using different amounts of sorbent.

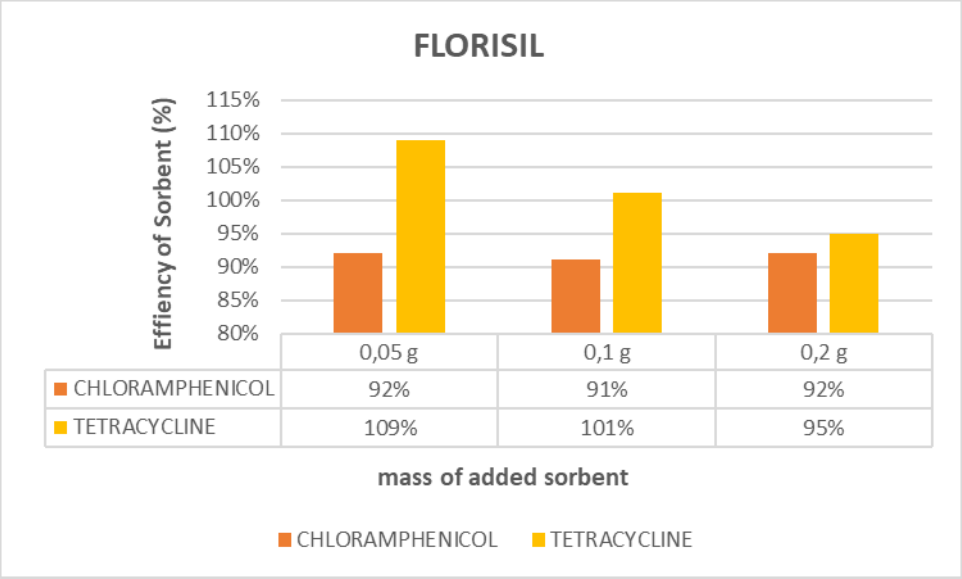

Figure 5. The influence of the mass of florisil as a sorbent on the extraction efficiency of antibiotics

The influence of the mass of florisil as a sorbent on the extraction Florisil is mostly used to retain fat and other non-polar compounds (Zhang et al., 2015). Nagata and Saeki (1986) have used florisil columns in the clean-up of ampicillin from fish tissues When analyzing samples containing chloramphenicol, florisil showed an equal efficiency for 0.05, 0.1 and 0.2 g of sorbent (92%), so extraction efficiency when using florisil does not depend on the mass of applied sorbent. When analyzing samples containing tetracycline, there is a decrease in extraction efficiency with an increase in the mass of florisil in the solution, with a difference in efficiency of 14% when applying 0.05 g and 0.1 g of sorbent. Based on these results, it can be seen that there is a big difference in efficiency when using different amounts of sorbent and in relation to its efficiency with chloramphenicol, and it is desirable to repeat the analysis under other conditions, for example by changing the length of extraction or centrifugation in order to further analyze the application of this sorbent in the analysis tetracycline and studies of the influence of other factors on the results of the analysis. Xu et al. (2021) examined usage of Primary Secondary Amine (PSA), C18, Graphitized Carbon Black (GCB), florisil, and ZrO_2 for were for the determination of 2

lincosamides and 13 macrolides in honey samples. Their results for extraction efficiency when using florisil as dSPE sorbent were between 40 and 100%, depending on antibiotic type.

When activated carbon was used as a sorbent, the results show that efficiency for tetracycline analysis was about 40% when the amount of added sorbent is 0.05 and 0.1g. For 0.1 g of added sorbent, the efficiency is 37%, which shows that the amount of added activated carbon does not affect its efficiency much.

Activated carbon shows a lower efficiency in the analysis of chloramphenicol, according to the results shown in figure 6.

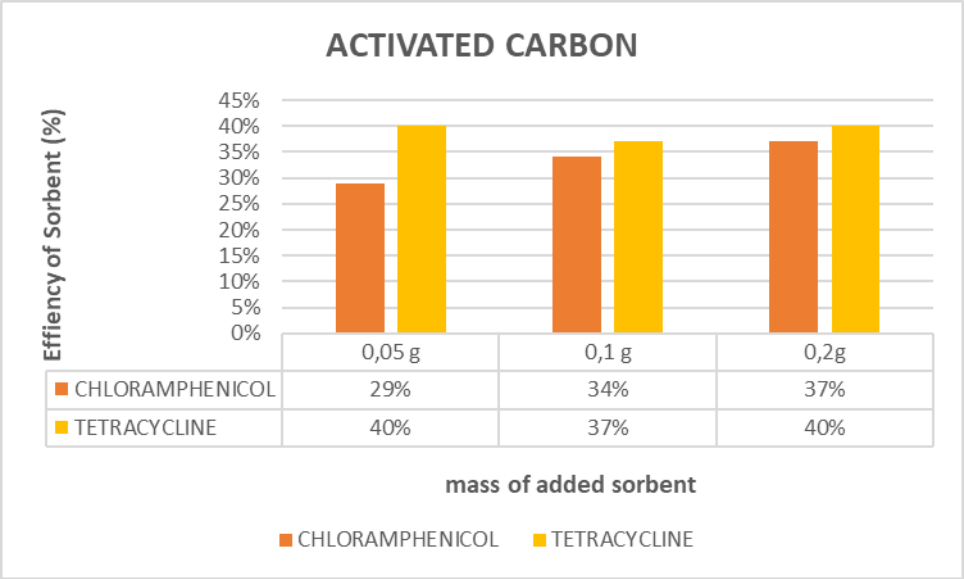

Figure 6. The influence of the mass of activated carbon as a sorbent on the extraction efficiency of antibiotics

The lowest efficiency (29%) is in case when 0.05 g of sorbent is added. By increasing the mass added sorbent, the efficiency also increases, i.e. with the addition of 0.1 g and 0.2 g of activated carbon, sorbent efficiency is 34 and 42%, respectively. It can be concluded that activated carbon is not suitable for sample preparation, because of its binding affinity for analyzed antibiotics. However, it can be used for tetracycline and chloramphenicol removal from various matrices. Lach (2019) analyzed the adsorption of chloramphenicol on commercially available activated carbon

and came to the conclusion that activated carbon can be used to remove chloramphenicol from a water sample. Also, Zhao et al. (2020) used newly synthesized activated carbon to remove tetracycline from water samples and concluded that this activated carbon can be successfully used to remove this antibiotic from water.

Conclusion

In this paper the efficiency of different sorbents for dSPE extraction of antibiotics was examined. Chloramphenicol and tetracycline are chosen because of their most common application both for human treatment and in food production. These antibiotics are often found in the environment as a result of inadequate use, and their analysis is of great importance.

C18 shows high efficiency when analyzing chloramphenicol and tetracycline. Efficiency values obtained for chloramphenicol analysis, with the increasing amount of sorbent were: 92, 92 and 96%, respectively. When analyzing tetracycline, there are some deviations in the results, so it can be seen that there is a difference in efficiency when using different amounts of sorbent.

When using florisil in the analysis of chloramphenicol, the obtained results show that the efficiency does not depend on the mass of added sorbent, while in the analysis of samples containing tetracycline, the efficiency decreases with the increase in the mass of the added sorbent.

In this experiment, activated carbon showed poor extraction efficiency of tetracycline and chloramphenicol, it binds a significantly larger amount of antibiotics compared to other sorbent, which was expected considering earlier studies of this sorbent, whereby binds chloramphenicol more than tetracycline. Lowest value for efficiency of extraction was obtained for activated carbon as a sorbent, in the analysis of chloramphenicol (29%). The activated carbon used in this experiment can be used for removal chloramphenicol and tetracycline from the samples, and with potential modifications it is possible improve the removal efficiency of these two antibiotics.

Acknowledgment

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (grant no 451-03-47/2023-01/ 200124).

Conflict-of-Interest Statement

The author did not declare any conflict of interest.

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