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DEVELOPMENT OF A MULTICLASS METHOD FOR 91 VETERINARY DRUGS IN PIG FAECES BY LIQUID CHROMATOGRAPHY HIGH-RESOLUTION MASS SPECTROMETRY

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Introduction

Veterinary drugs are widely used to treat diseases of livestock [1]. They are mainly excreted from animal body by the digestive and urinary system which leads to residue presence in urine and faeces samples and, therefore, could cause environmental issues, also related to their agronomic application as fertilisers. Accordingly, the concerns about these practices are increasing due to their possible contribution to the antibiotic resistance (AR) phenomena. At present, the correlation between the presence of antibiotics in animal faeces and AR is not well known [2].

As matter, a multiresidue method was developed for detecting and quantifying 91 veterinary drugs in pig faeces, encompassing twelve antibiotic and antiparasitic families (amphenicols, cephalosporins, penicillins, diamino-pyrimidine, lincosamides, macrolides, pleuromutilins, quinolones, rifamycins, sulphonamides, tetracyclines, benzimidazoles and avermectins) and other minority groups or single compounds.

Experimental

The samples were firstly extracted with a mixture of acetonitrile (ACN)/water containing formic acid and EDTA and, then, with the same mixture (ACN/Water) containing ammonia, followed by the filtration of reunited extracts with NH_2 SPE cartridge. After evaporation, the dry extracts were dissolved in an acetonitrile/ammonium acetate solution. The analysis was carried out using liquid chromatography coupled to a Q-Orbitrap mass spectrometer (LC-Q-Exactive, Themo Scientific, San Jose, CA, USA) using both positive and negative electrospray ionization mode. The instrumental conditions were those described in Moretti et al. [3] with slight modifications.

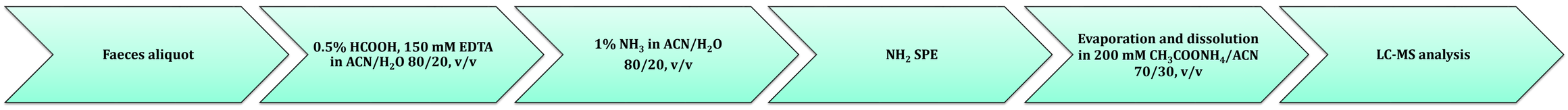

Figure 1 - ***Sample preparation***

Results and discussion

The high concentration of interferent substances in faeces and the variety of analytes properties forced to investigate deeply every step of the sample preparation resulting in a large number of experiments to develop the final protocol. Figure 2 shows the performances observed for sulphonamides, an important class of antibiotics, using three purification approaches. On the whole, the best results were obtained applying a non retentive SPE (NH_2 cartridge) with recoveries generally higher than 60% and repeatibilities lower than 10%. Figure 3 demonstrates the influence of the extraction mixture volume on tetracycline accuracies.

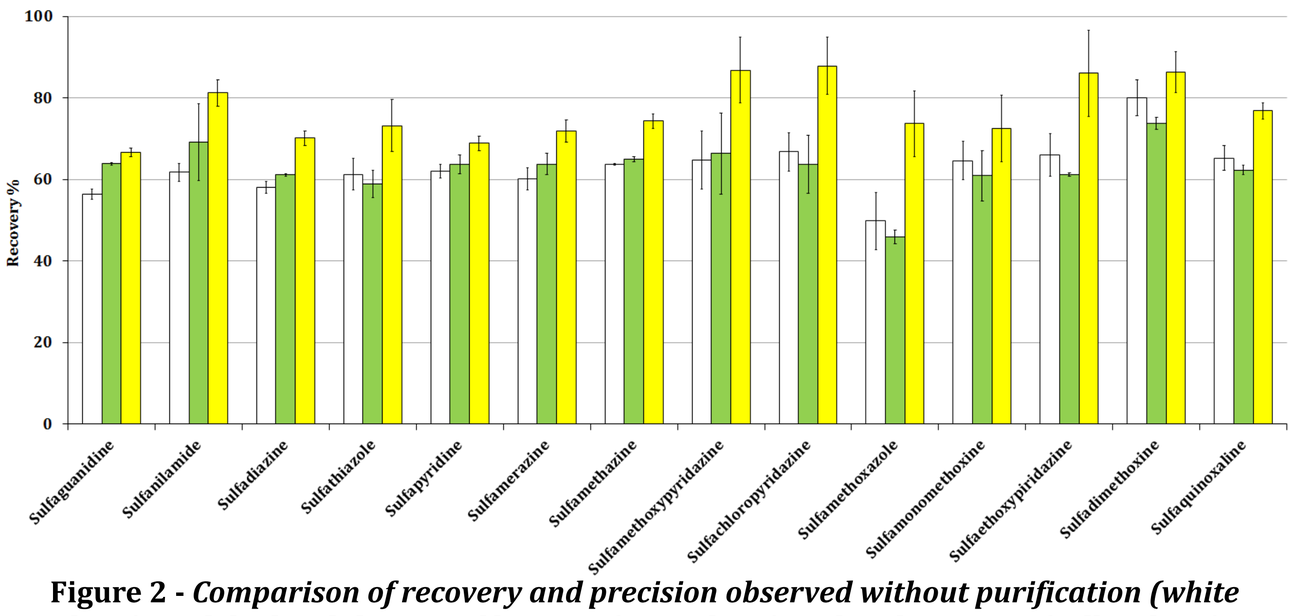

Figure 2 - ***Comparison of recovery and precision observed without purification (white bars), with hydrophilic PTFE filtration (green bars) and with NH_2 SPE (yellow bars)***

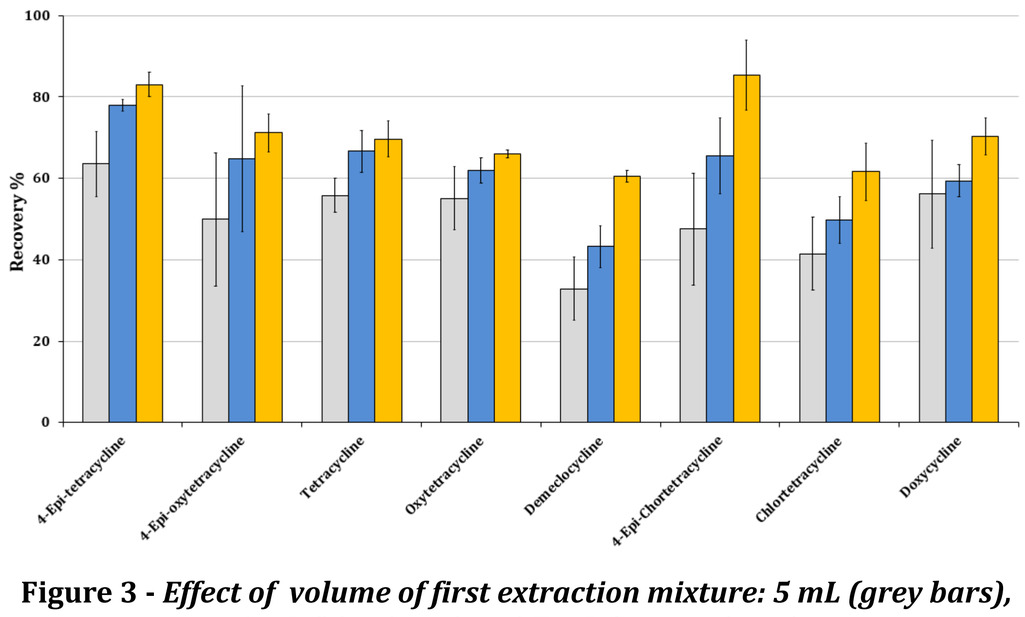

Figure 3 - ***Effect of volume of first extraction mixture: 5 mL (grey bars), 6 mL (blue bars) and 7 mL (orange bars)***

Conclusions

The analysis of veterinary drugs in faeces is a non-invasive strategy to monitor the antibiotic use in animal breeding and to obtain knowledge about their environmental diffusion.

The preliminary validation data were satisfactory [4].

References

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