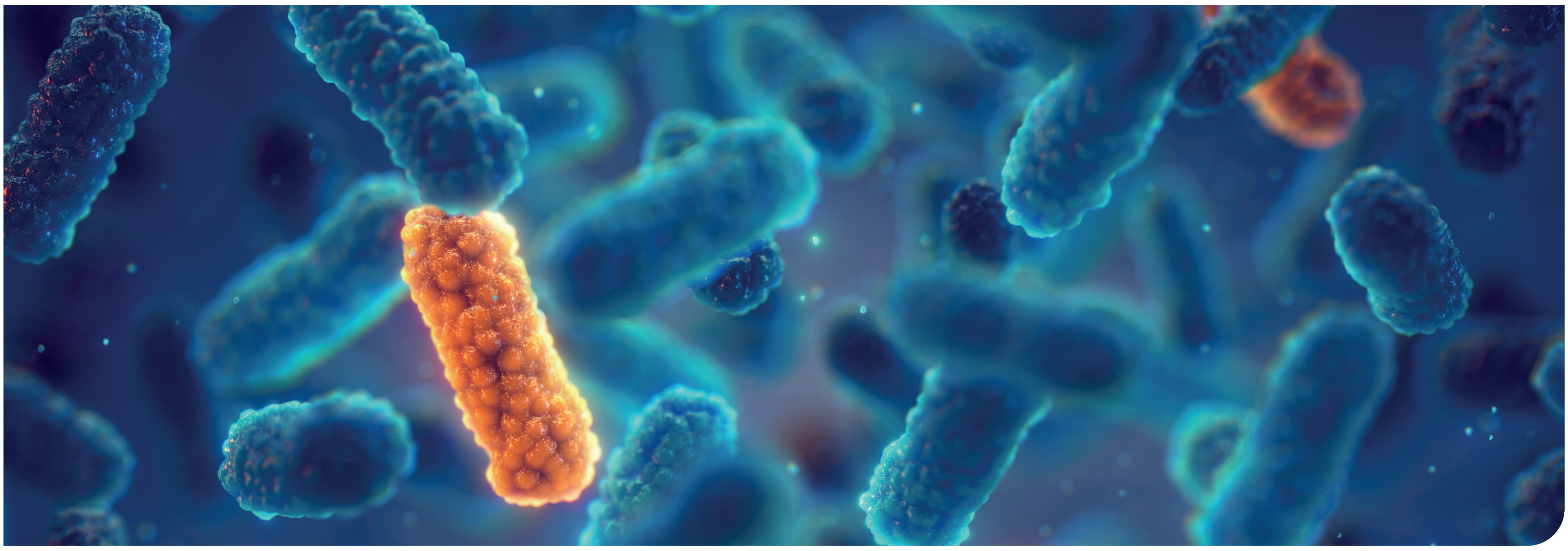
DEVELOPMENT OF ANALYTICAL METHODS TO ASSESS ANTIMICROBIAL CONTAMINATION IN THE ENVIRONMENT AND ITS TRANSFER IN THE FOOD CHAIN



The extensive use of antimicrobials (AMs) in agriculture has led to the occurrence of residual drugs in different environmental matrices such as animal manure (Zalewska et al., 2021), soils (Hang et al., 2021) and vegetables (Kang et al., 2013) frequently consumed by humans, among others. This could pose a potential threat to human health, not only because of the possible effects after ingestion but also because the transmission of AM-resistant genes could occur (Jadeja & Worrich, 2022).

In this sense, two accurate sample preparation procedures were developed and validated for the simultaneous analysis of sulfonamides (SAs) and tetracyclines (TCs) in four of the most widely consumed vegetables (lettuce, onion, tomato, and carrot) in Europe (Vergara-Luis, Báez-Millán, et al., 2023). The evaluated protocols were based on QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) for extraction and subsequent clean-up by SPE (solid phase extraction) or dispersive SPE. Parameters affecting both extraction and clean-up were carefully evaluated and selected for accuracy of results and minimal matrix effect (Figure 1).

Overall, apparent recoveries were above 70 % for most of the target analytes with both analytical procedures, and adequate precision (RSD < 30 %) was obtained for all the matrices. The procedural limits of quantification (LOQ_{PRO}) values for SPE clean-up remained below 4.4 μg·kg^{-1} for TCs in all vegetables except for chlortetracycline (CTC) in lettuce (11.3 μg·kg^{-1}) and 3.0 μg·kg^{-1} for SAs, with the exception of sulfadiazine (SDZ) in onion (3.9 μg·kg^{-1}) and sulfathiazole (STZ) in carrot (5.0 μg·kg^{-1}). Lower LOQ_{PRO} values (0.1-3.7 μg·kg^{-1}) were obtained, in general, when dSPE clean-up was employed. Both methods were applied to twenty-five market vegetable samples from ecological and conventional agriculture and only sulfamethazine (SMZ) and sulfapyridine (SPD) were detected in lettuce at 1.2 μg·kg^{-1} and 0.5 μg·kg^{-1}, respectively.

Moreover, an accurate analytical method was also developed for the simultaneous analysis of twenty-four AMs in soil:compost and animal manure samples by means of Ultra-High Performance Liquid Chromatography coupled to a triple-quadrupole mass spectrometer (UHPLC-QqQ) (Vergara-Luis, Bocayá, et al., 2023).

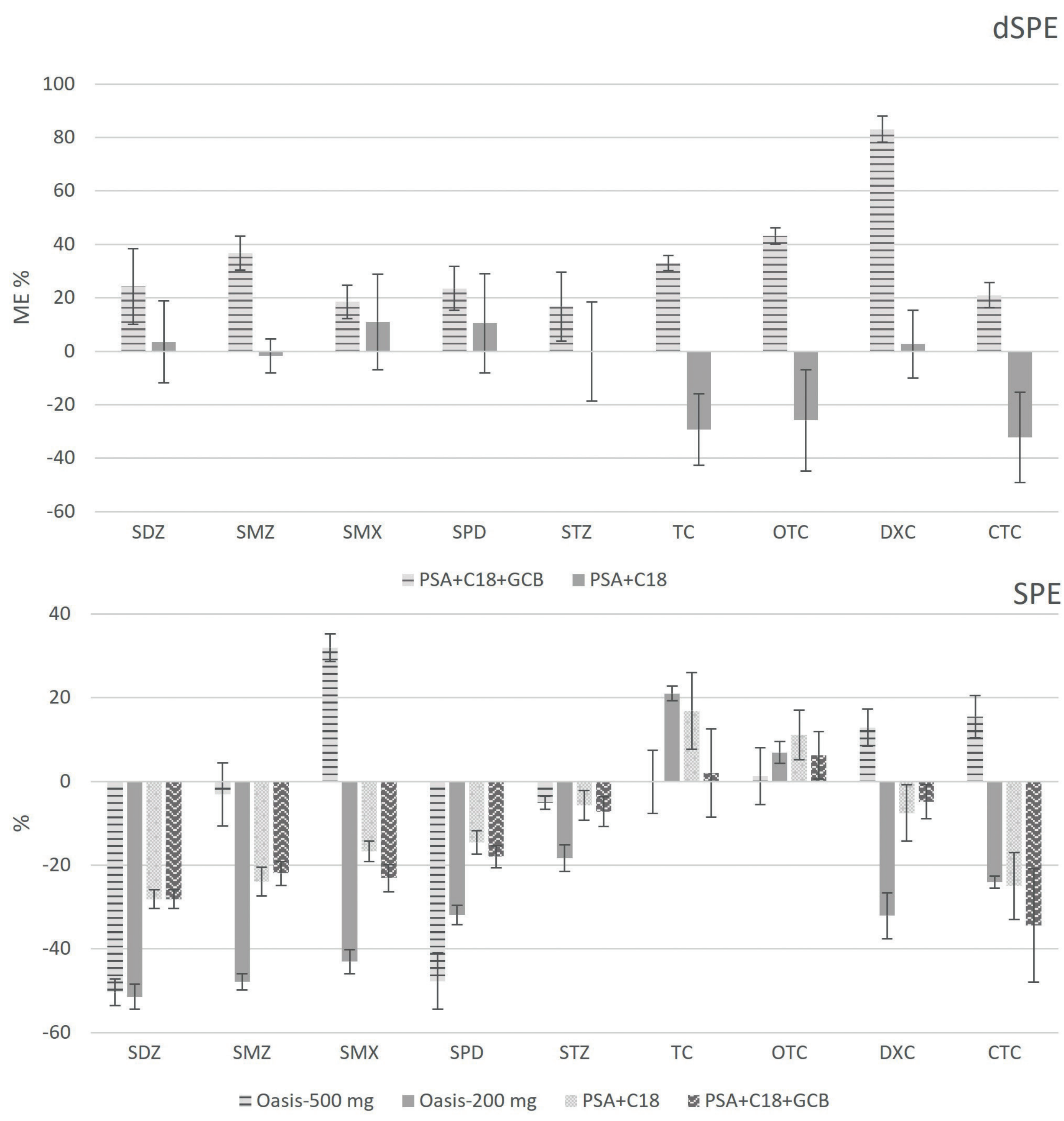


Figure 1. Matrix effect (ME) % (n=3) at the detection for the target analytes in the four vegetable matrices with SPE and dSPE clean-up approaches.

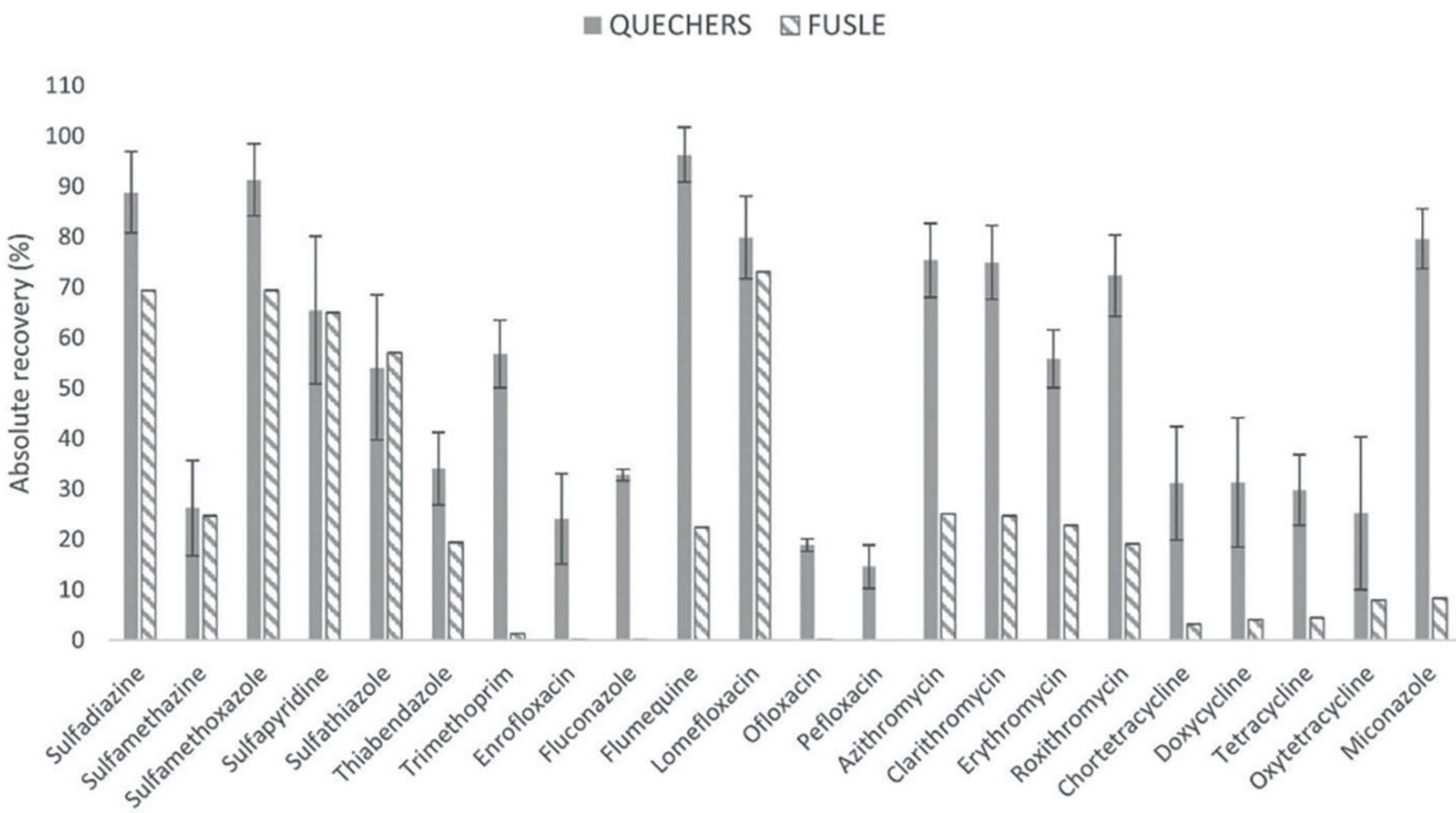


Figure 2. Absolute recoveries for each target analyte (n=4) after performing QuEChERS-SPE or FUSLE-SPE extraction.

For this purpose, the effectiveness of two extraction techniques (i.e., focused ultrasound solid-liquid extraction (FUSLE) and QuEChERS was evaluated and the clean-up step using solid phase extraction (SPE) was also thoroughly studied (Figure 2).

The method was successfully validated at 10 $\mu g \cdot kg^{-1}$, 25 $\mu g \cdot kg^{-1}$ and 50 $\mu g \cdot kg^{-1}$ showing adequate trueness (70-130 %) and repeatability (RSD<30 %), with few exceptions. Procedural limits of quantification (LOQ_{PRO}) were determined for soil:compost (0.45 to 7.50 $\mu g \cdot kg^{-1}$) and manure (0.31 to 5.53 $\mu g \cdot kg^{-1}$) samples. Pefloxacin could not be validated at the lowest level since $LOQ_{PRO} \geq 10$ $\mu g \cdot kg^{-1}$. Sulfamethazine (7.9 ± 0.8 $\mu g \cdot kg^{-1}$), danofloxacin (27.1 ± 1.4 $\mu g \cdot kg^{-1}$) and trimethoprim (4.9 ± 0.5 $\mu g \cdot kg^{-1}$) were detected in soil samples; and tetracycline (56.8 ± 2.8 $\mu g \cdot kg^{-1}$), among other AMs, in the plants grown on the surface of the studied soil samples. Similarly, SAs, TCs and fluoroquinolones (FQs) were detected in sheep manure in a range of (1.7± 0.3) - (93.3± 6.8) $\mu g \cdot kg^{-1}$. Soil and manure samples were also analysed through UHPLC coupled to a high-resolution mass-spectrometer (UHPLC-qOrbitrap) in order to extent the multitarget method to suspect screening of more than 22,281 suspects. A specific transformation product (TP) of sulfamethazine (formyl-sulfamethazine) (Figure 3) was annotated at 2a level in manure samples, among others.

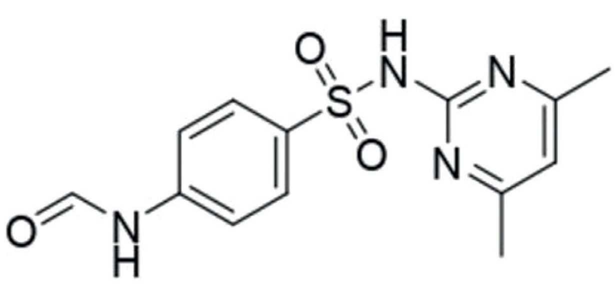


Figure 3. Chemical structure of the identified TP, formyl-sulfamethazine.

This work contributes to the efforts that have been made in the last decade to develop analytical methods that allow multitarget analysis of a wide variety of AMs, including TPs, in complex environmental matrices, which is a complex task due to the diverse physicochemical properties of the AMs. Furthermore, the results of this work not only demonstrated the presence of AMs in the environment and the transference of these pharmaceuticals in the manure-soil-plant/vegetable chain, which puts pressure on bacteria to create resistance, but also showed the presence of their TPs, the effects of which are still unknown.

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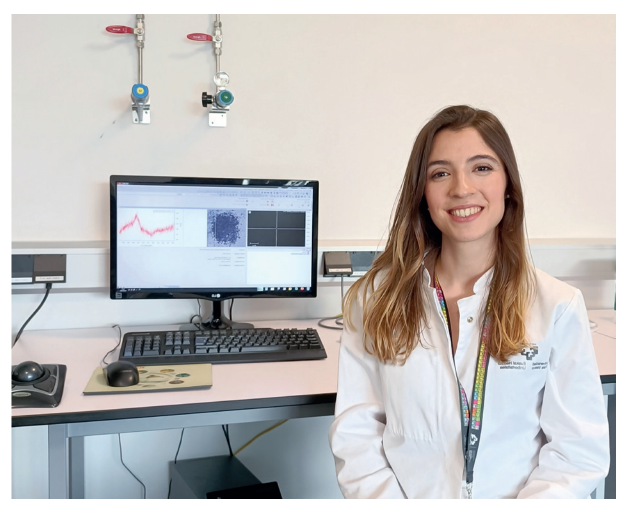

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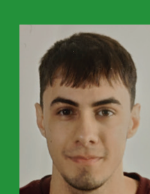
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