Multitarget and suspect-screening of antimicrobials in vegetables samples: Uptake experiments and identification of transformation products

I. Vergara-Luis [a,b,*], M. Jin [a], J.C. Baez-Millán [a], B. González-Gaya [a,b], I. Ijurco [c], M. Lacuesta [c], M. Olivares [a,b], A. Prieto [a,b]

[a] Department of Analytical Chemistry, Faculty of Science and Technology, University of the Basque Country (UPV/EHU), Leioa, Basque Country, Spain
[b] Research Centre for Experimental Marine Biology and Biotechnology (PIE), University of the Basque Country (UPV/EHU), Plentzia, Basque Country, Spain
[c] Department of Plant Biology and Ecology, Faculty of Pharmacy, University of the Basque Country (UPV/EHU), Vitoria-Gasteiz, Basque Country, Spain

ARTICLE INFO

ABSTRACT

This work provided an accurate analytical method to perform a multitarget analysis of a variety of antimicrobials (AMs) including sulfonamides, tetracyclines, macrolides, fluoroquinolones and quinolones, one imidazole and one nitroimidazole, one triazole, one diaminopyridine and one derivative of *Penicillium stoloniferum* in vegetables. The analysis is performed using liquid-chromatography coupled to a low-resolution triple quadrupole mass spectrometer (UHPLC-MS/MS) to detect the target analytes or coupled to a high-resolution q-Orbitrap (HRMS) to monitor the formed transformation products (TPs). Both instruments were compared in terms of limits of quantification and matrix effect at the detection. The method was applied to determine the presence of AMs in organic and non-organic vegetables, where sulfadiazine and mycophenolic acid were detected. On the other hand, the transference of four AMs (trimethoprim, sulfamethazine, enrofloxacin, and chlortetracycline) from soils to lettuces was evaluated through controlled uptake experiments. The choice of AMs was based on the classification into different families, and on the fact that those AM families are the most frequently detected in the environment. In this case, each of the AMs with which the soils were contaminated were found in the exposed lettuces. Moreover, in both studies, specific TPs of the AMs were identified, posing the necessity of assessing their effects in relation to food and human safety.

1. Introduction

The growing global population and the progress of society have resulted in the widespread use of chemicals, which serve to enhance our everyday lives and promote human welfare. However, the continued use of chemicals has led to their accumulation in the environment, resulting in drugs such as antimicrobials (AMs) being considered environmental pollutants (García et al., 2020).

AMs have been found in numerous environmental samples, including water (Valcárcel et al., 2011), sludge (Li et al., 2013), animal manure (Zalewska et al., 2021), soil (Hang et al., 2021), and even vegetables (Kang et al., 2013) as a result of their entry into the food chain. However, AMs are frequently reported near the limits of quantification in scientific literature due to their high biodegradability under light or temperature conditions (Cycoń et al., 2019). Accordingly, more and more scientific studies are focusing the search and determination of transformation products (TPs) (Sunyer-Caldú & Diaz-Cruz, 2021; Tadić

et al., 2019). The main observations of those studies suggest that the concentration of TPs in vegetable samples might actually exceed the levels of their precursor compounds. For instance, Tadić et al. reported a five-fold higher concentration of trimethoprim 304 (TMP304), a degradation product of TMP, in a lettuce sample compared to its precursor (Tadić et al., 2019), while different TPs of sulfonamides (SAs) were found by Sunyer et al. (Sunyer-Caldú & Diaz-Cruz, 2021) in lettuces irrigated with reclaimed water.

The presence of AMs in even low concentrations can be detrimental to both human and ecosystem health. This includes the potential spread of AM resistance, allergic reactions, toxicity, and more (Jadeja & Worrich, 2022). Therefore, it is essential to establish a reliable analytical methodology for effectively monitor both AMs and TPs in complex environmental samples, such as vegetables.

The high concentration of pigments and cellulose in vegetable samples poses a significant analytical challenge for the analysis of AMs, as these major interferences can significantly impact the sensitivity of the

---

method (Anumol et al., 2017). Liquid chromatography tandem mass spectrometry (LC-MS/MS) enables the comprehensive analysis of multiclass AMs within complex matrices after an effective sample pretreatment to remove any interference while preserving the target compounds. (Dasenaki & Thomaidis, 2015). Nowadays, ultra high-performance LC (UHPLC) has overtaken high-performance LC (HPLC) in terms of resolution and speed of analysis (Rodriguez-Aller et al., 2013) and it is often coupled to low resolution triple quadrupole (QqQ) mass analyser (Mijangos et al., 2019) when multiresidue drug analysis at regulatory control concentration levels is required. In order to extend the analysis of unknown TPs (Jongedijk et al., 2023), a growing number of studies are employing high-resolution MS (HRMS) mass analysers such as q-Orbitrap (Castellani et al., 2023) and/or Time-of-Flight (TOF) (Varenina et al., 2022). Regarding the ionisation, electrospray ionisation (ESI) (Sun et al., 2016) is often used for low- and high-resolution analysis.

While recent research has made significant strides in the advancement of methodologies enabling the simultaneous analysis of AMs and TPs in various matrices, the analysis is still frequently constrained to specific AM families (Barron et al., 2008; Meng et al., 2017). In such cases, when multiple AM groups are studied together, the accuracy requirements of the regulations are not fully satisfied (da Silva et al., 2020).

Within this context, the present work aimed to extend the method previously developed in the research group for SAs and tetracyclines (TCs) determination (Vergara-Luis et al., 2023) to cover the simultaneous analysis of five SAs, four TCs, four macrolides (MCs), nine fluoroquinolones and quinolones (FQs), one imidazole (IM) and one nitroimidazole (NIM), one triazole (TZ), one diaminopyridine (DAP) and one derivative of *Penicillium stoloniferum* (DP) in vegetables (lettuce, onion, tomato, and carrot) frequently consumed by humans. In order to achieve this goal, we fully studied the effectiveness of the clean-up protocol and we compared the sensitivity and selectivity of the UHPLC-MS/MS and UHPLC-HRMS instruments. Furthermore, concerning to HRMS, two acquisition modes (Discovery and Confirmation) were experimented with in order to strike a balance between the number of compounds detected and the sensitivity and suitability required for suspect screening analysis. The analytical methods were used to identify AMs in several vegetable matrices (lettuce, onion, tomato, and carrot) and to investigate the accumulation and degradation of AMs in lettuce samples cultivated in polluted soils.

2. Experimental procedure

2.1. Reagents and materials

The physicochemical properties for the target AMs and surrogate standards and their distributors are gathered in **Table S1**. Individual solutions for all of them were monthly prepared at 1000–3000 mg·kg^{-1} in UHPLC-grade methanol (MeOH, 99.9 %, Scharlau, Sentmenat, Catalonia, Spain), UHPLC-grade acetonitrile (ACN, 99.9 %, Avantor Performance Materials, Gliwice, Silesia, Poland) or dimethyl sulfoxide (DMSO, Panreac AppliChem, Darmstadt, Germany) (see **Table S1**). The individual stock solutions of FQs were prepared adding drops of NaOH (2 M) (99 %, Merck, Darmstadt, Hesse, Germany) as reported in a previous work (Vergara-Luis et al., 2023). Further combined dilutions were weekly prepared in ACN at 100 mg·kg^{-1} and 5 mg·kg^{-1} for sample spiking. Most concentrated solutions (1000–3000 mg·kg^{-1} and 100 mg·kg^{-1}) were stored at −20 °C, while 5 mg·kg^{-1} ones were kept at 4 °C, using silanised amber vials (Burhenne et al., 1999).

The sample extraction procedure employed the following salts: NaCl (100 %) acquired from PanReac AppliChem (Castellar del Vallés, Catalonia, Spain), anhydrous citric acid H$_3$Cit (99.5 %) and anhydrous Na$_2$HPO$_4$ (98 %) from Scharlau and anhydrous Na$_2$SO$_4$ (99 %) from Merck. UHPLC-grade ACN was set as the extraction solvent.

The dispersive clean-up step (dSPE) method for vegetable samples involved the use of Primary Secondary Amine (PSA), Bondesil-C$_{18}$ (40 μm, Agilent Technologies, Santa Clara, CA, EEUU) and Graphitised Carbon Black (GCB) (37–125 μm, Superclean ENVI-Carb, Merck) sorbents; whereas Oasis HLB cartridges (200 mg, 6 cc, 30 μm) purchased from Waters (Milford, Massachusetts, USA) were employed for the clean-up of soil samples. A citrate buffer consisting of an aqueous solution of anhydrous NaH$_2$Cit (99 %) and Na$_2$HCit·1.5H$_2$O (99 %) (Honeywell Fluka, Charlotte, North Carolina, USA) was also used in soil analysis. Oxalic acid (100 %, Merck) was used in the final extract reconstitution.

During the sample treatment procedure, a Multi Reax shaker by Heidolph (Schwabach, Bavaria, Germany) and a 5840R centrifuge by Eppendorf (San Sebastián de Los Reyes, Madrid, Spain) were used.

2.2. Sample treatment procedure: Extraction and clean-up

This study extends the previously developed method by the research group (Vergara-Luis et al., 2023) to simultaneously determine twenty-seven AMs in vegetables (lettuce, onion, tomato, and carrot). Briefly, fresh, crushed and homogenised vegetable (lettuce, onion, tomato or carrot) samples (10 g) were spiked with 200 μL of a 5 mg·kg^{-1} stock solution, vortexed (2000 cycles·min^{-1}, 10 min) and kept in the darkness for 30 min at room temperature. ACN (10 mL), a ceramic homogeniser and the salts (4 g anhydrous Na$_2$SO$_4$, 1 g NaCl, 0.5 g anhydrous H$_3$Cit and 0.049 g anhydrous Na$_2$HPO$_4$) were added. The mixture was then shaken manually and degasified by opening the centrifuge tube until no gas was released. All samples were vortexed (2000 cycles·min^{-1}, 8 min) and centrifuged (4000 cycles·min^{-1}, 5 min) at 10–15 °C.

The presence of co-eluting elements in the matrix can lead to interferences during analysis, making it essential to carry out a clean-up procedure. For that purpose, dSPE approach was employed and basing on the literature and previous experience of the research group (He et al., 2018; Vergara-Luis et al., 2023), two sorbent combinations were evaluated: PSA (10 mg) and C$_{18}$ (25 mg), with or without GCB (2.5 mg) addition, together with 150 mg anhydrous Na$_2$SO$_4$ in all the cases. Under optimal conditions, an aliquot of 1 mL of the extractant was transferred to a 50 mL centrifuge tube containing 10 mg PSA, 25 mg C$_{18}$ and 150 mg Na$_2$SO$_4$. The mixture was then vortexed (2000 cycles·min^{-1}, 1 min) and centrifuged (4000 cycles·min^{-1}, 5 min) at 10–15 °C. Aliquots of 500 μL were reconstituted in 1 mL of 1:1 (v/v) ACN:oxalic acid (aq., 0.01 mol·L^{-1}, pH 2) and filtered through 0.22 μm polypropylene filters (Clarify-PP, Phenomenex, Torrance, California, USA) before UHPLC-MS/MS and UHPLC-HRMS analysis.

2.3. Analysis

2.3.1. UHPLC-MS/MS

An Agilent 1290 Infinity II UHPLC coupled to an Agilent 6430 Triple Quad tandem mass-spectrometer (QqQ) (Agilent Technologies) was used for multitarget analysis. Previous research conducted by our group has already documented the mobile phase, chromatographic column, and various detector parameters used (Vergara-Luis et al., 2023; Vergara-Luis et al., 2023) which are indicated in the **section 1.** of the supplementary material.

2.3.2. UHPLC-HRMS

A Thermo Scientific Dionex UltiMate 3000 UHPLC coupled to a Thermo Scientific Q Exactive Focus quadrupole-Orbitrap mass spectrometer (UHPLC-q-Orbitrap) equipped with a heated ESI source (HESI, Thermo-Fisher Scientific, CA, USA) was used to perform a suspect analysis of more than 22,278 suspects. To achieve this, we evaluated the chromatographic separation resolution and sensitivity using two columns (i.e., C$_{18}$ and C$_{18}$ polar columns) and three organic mobile phases (i.e., ACN with 0.1 % of HCOOH, MeOH with 0.1 % of HCOOH and MeOH with 0.1 % of oxalic acid). The sensitivity of the detection was tested by the optimisation of HESI conditions: (i) capillary temperature

(200 °C, 320 °C and 400 °C) and, (ii) spray voltage (2.5 kV, 3.2 kV and 4 kV). Moreover, full scan – data dependent MS2 (Full MS-ddMS2) discovery and confirmation acquisition modes were evaluated for further AM identification.

According to the results in section 3.1, the ACE UltraCore XB-C$_{18}$ chromatographic column (2.1 mm x 150 mm, 1.7 μm) with a pre-filter (2.1 mm ID, 0.2 μm) from Phenomenex was set as optimal for the analysis and Milli-Q water (0.1 % HCOOH) (A) and MeOH (0.1 % HCOOH) (B) were used as mobile phases. The HESI source parameters were set to 4 kV for the spray voltage and 400 °C for the capillary temperature. Method details and parameters are included in section 2. of the supplementary material.

2.4. Method validation

In this work, UHPLC-MS/MS and UHPLC-HRMS analysis techniques were evaluated in terms of linearity, precision (instrumental and procedural repeatability), instrumental (LOQ$_{INS}$) and procedural (LOQ$_{PROC}$) limits of quantification and matrix effect at the detection.

LOQ$_{INS}$ were calculated using a thirteen-point external calibration curve (0.25–200 μg·kg^{-1}), as the lowest external calibration point with RSD % and a systematic error in relation to the theoretical value below 30 %. For that aim, the calibration curve points between 0.25 and 25 μg·kg^{-1} concentration levels were measured in triplicate. This data was also employed to estimate instrumental repeatability (in the same day) and intermediate repeatability (in different days). Linearity ranges were defined considering the determination coefficients (r^2) of the calibration curves built between LOQ$_{INS}$ and upper limit. The latter was established as the highest concentration possible that provided r^2 value closest to one, avoiding quadratic fitting of the calibration curves.

LOQ$_{PROC}$ values, defined as the lowest concentration fulfilling the criteria of an RSD % and a systematic error lower than 30 %, were obtained by spiking vegetable extracts (final extracts, after been submitted to the whole procedure) at different concentration levels (1, 2.5, 5, 7.5, 10, 15, 25 and 50 μg·kg^{-1}) and analysing them in triplicate.

Matrix effect at the detection was determined using Eq. (1) as described in previous works (Vergara-Luis et al., 2023).

$$\text{Matrix effect } (\%) = \left(\frac{\text{Area}_{\text{sample}}}{\text{Area}_{\text{Reference}}} - 1 \right) \text{x } 100 \qquad (1)$$

Trueness was determined using isotopically labelled compounds and matrix-matched calibration approach (using a six-point calibration curve in the range of 1–75 μg·kg^{-1} prepared in each of the four vegetable matrices). The repeatability of the procedure was also evaluated in terms of RSD %, using three replicates of each real sample processed in the same day.

Finally, for suspect analysis, instrumental (LOI$_{INS}$) and procedural (LOI$_{PROC}$) limits of identification were calculated using the same concentration levels selected for the calculation of the LOQs. LOIs were estimated as the lowest concentration for which the experimental and theoretical MS2 spectra match was equal or greater than 70 % in at least two of the three replicates for each concentration level and the retention time difference was less than ± 0.1 min.

2.5. Data treatment

2.5.1. UHPLC-MS/MS

The raw files obtained from the UHPLC-MS/MS were subjected to data processing using the Agilent MassHunter Workstation software (Quantitative Analysis for QQQ, version 10.0) by Agilent. Analyte identification and quantification criteria were established according to guidelines detailed in the Council Directive 96/23/EC (Commission Decision, 2002). The presence of the compound was effectively verified by comparing its retention time with a reference standard, alongside the detection of the two most distinctive transitions for each target compound.

2.5.2. UHPLC-HRMS: Target analysis and suspect screening

Even though the UHPLC-HRMS used a non-target method for acquisition, the data was processed using two approaches: target analysis and suspect screening. For the UHPLC-HRMS target analysis, TraceFinder 5.1 (Thermo-Fischer Scientific) software was used to identify and quantify the studied AMs taking into account the retention time, precursor and product ions of the selected and previously known AMs (Table S2). A 5 ppm variability was considered acceptable for monoisotopic mass and fragments, and a 70 % fit for theoretical isotopic pattern.

For the UHPLC-HRMS suspect screening, the Compound Discoverer 3.2 (Thermo-Fisher Scientific) software was used. For candidates' identification, firstly ACN blanks were used as reference for noise elimination. As for the peak selection criteria, the "peak intensity" filtering criterion was set at 10,000 to consider only candidates with a minimum peak area equal to or greater than 10,000. In addition, the Lorentzian shape of the chromatographic peaks and a mass error of less than 5 ppm were established as mandatory criteria for further annotation. Different mass lists, such as the COMPTOX list, which includes AMs and TPs, and those generated with the BioTransformer 3.0 software were used, to broaden the search for metabolic TPs to 22,278 compounds in total. Only the features included in the mass lists were considered and a spectral match between the experimental and the theoretical MS1 of more than the 70 % was required. Furthermore, peaks with chromatographic areas three times larger than the blanks and with a relative standard deviation (RSD %) lower than 30 % within the three injection replicates were taken into account. Moreover, due to the specific isotopic profiles of molecules containing O, N, Cl, Br, S and/or F, the analysis was limited to compounds containing these atoms. mzCloud database (https://www.mzcloud.org/) was used for MS2 comparison and a threshold value of 70 % or higher was considered for positive identification. When mass spectras were not available in the database mentioned above, Mass Frontier Spectral Interpretation Software (Thermo-Fisher Scientific) was used for *In-silico* fragmentation. Retention times of the candidates must match those predicted by the Retention Time Index (RTI) platform (https://rti.chem.uoa.gr/) and the candidates were rejected or accepted depending on whether or not there was a statistical difference with the estimated value within the uncertainty of the built model (only box 1 and box 2 candidates were considered). For compounds' annotation the confidence level established by Schymanski et al. (Schymanski et al., 2014) was used and, in this work, only those candidates annotated at a confidence level 1 (candidate confirmed by MS1, MS2 and retention time), 2 (candidate confirmed by MS^2 library matching (Level 2a) or diagnostic MS^2 *in-silico* fragmentation (Level 2b) when no standard or experimental MS^2 database is available) and 3 (candidate confirmed by MS1 and *in-silico* MS2, being all the candidates structural isomers) were reported.

Regardless of the analysis mode, when it was required, statistical analysis was carried out performing an ANOVA test in Excel (2021 version).

2.6. Method application: Detection of AM contamination and lettuce uptake experiment

The validated method was applied to two different case studies:

i. The analysis of fourteen lettuces, three onions, three tomatoes and ten carrots from organic (seventeen) and non-organic (thirteen) agriculture, purchased in local markets in the Basque Country.
ii. The transfer of four AMs (trimethoprim, sulfamethazine, enrofloxacin, and chlortetracycline) from soils to lettuces and the evaluation of their degradation in both matrices. For this purpose, universal soil substrate and seeds of the commercial lettuce cultivar 'Batavia' (*Lactuca sativa* var. longifolia, Vilmorin seeds), one of the

most widely used in some areas of Europe (Ryder, s. f.), were used. Six pots, each containing 180 g of universal substrate, were prepared for each of the studied AMs. Three seeds were sown per pot, previously sterilised in order to ensure that at least one plant corresponded to each pot. All pots were kept in a growth chamber (Ibercex®), under controlled conditions of 23 °C/18 °C (day/night) temperature, 70/80 % (day/night) relative humidity, 450 μmol m^{-2} s^{-1} of photosynthetically active radiation (hereinafter, PAR), and a 14-hour photoperiod. Throughout their development, the plants were irrigated with distilled water three times a week. Two weeks after sowing, the number of plants per pot was homogenised to one, eliminating the remaining plants. Twenty-five days after sowing, the studied AMs were applied to the soil substrate, individually, at a concentration of 1 mg·kg^{-1} (three replicates per AM and three blanks were prepared). Soil and lettuce samples were taken in the first and third week after spiking and were analysed through UHPLC-MS/MS and UHPLC-HRMS. For soil analysis the method described in a previous work was applied (Vergara-Luis et al., 2023).

3. Results and discussion

3.1. UHPLC-HRMS: Setting up the chromatographic conditions

3.1.1. Chromatographic column and mobile phase

The chromatographic column (C$_{18}$ and C$_{18}$ polar columns) and mobile phase (ACN with 0.1 % of HCOOH, MeOH with 0.1 % of HCOOH and MeOH with 0.1 % of oxalic acid) were optimised to obtain the maximum chromatographic peak area and optimal chromatographic separation of the selected compounds. In a previous work (Vergara-Luis et al., 2023), the presence of oxalic acid has been reported to avoid chromatographic peak tailing of TCs, therefore, its use as a mobile phase component has been evaluated in this work. The chromatographic peak areas of the target AMs (except miconazole with was latter included in the method) injected in each selected column and mobile phase are shown in Figure S1.

The lowest chromatographic peak areas were obtained with the column C$_{18}$ polar and ACN with 0.1 % HCOOH as mobile phase. Opposite, the highest peak areas were observed for eight AMs (see Figure S1) when the organic mobile phase polarity was increased with MeOH. This last mobile phase in C$_{18}$ column retrieved better results for eleven compounds in comparison to the polar column whilst when oxalic acid was included only four AMs (flumequine, oxytetracycline, thiabendazole and sulfamethazine) showed a significant signal improvement. Regarding the retention times, the shorter times were recorded when the C$_{18}$ polar column was used for both high and low molecular weight compounds. Therefore, considering the more suitable results in terms of chromatographic separation, elution time and peak shape of the target AMs, the C$_{18}$ column was set as optimal, using MeOH with 0.1 % HCOOH as mobile phase.

3.1.2. Source parameters

To optimise the source parameters, three different capillary temperatures (200 °C, 320 °C, and 400 °C) were studied while maintaining a spray voltage of 3.2 kV for the target AMs, except for miconazole. Once the optimum capillary temperature was selected, three different voltages (2.5 kV, 3.2 kV and 4 kV) were evaluated.

As it can be observed in Figure S2, the less intensive signals for the target analytes were obtained when the capillary temperature was set at 200 °C. Statistically comparable results were recorded with 320 °C and 400 °C, however, at 320 °C SAs and FQs showed a lower ionisation. Therefore, the capillary temperature was set at 400 °C.

Regarding the spray voltage (Figure S3), although no statistical differences were observed at the three tested values, the highest signal intensities for seventeen of the twenty-six AMs were obtained at a voltage of 4 kV. Thus, 4 kV was the selected as the optimal voltage.

3.2. Evaluation of the clean-up step

For the clean-up step, the addition of GCB (2.5 mg) to the dSPE sorbents PSA (10 mg), C$_{18}$ (25 mg) and Na$_2$SO$_4$ (150 mg) was evaluated in terms of matrix effect at the detection and recoveries of the target analytes. The analysis was performed using UHPLC-MS/MS. According to the results (see Figure S4), no statistical differences for recovery values were noticed when GCB was used (43–118 % with vs 44–121 % without GCB). However, GCB presence led to a higher positive matrix effect for some compounds, being especially noticeable for TCs. Therefore, the use of GCB was discarded in further assays and we proceeded to determine the adequacy of instrumental set-up.

3.3. UHPLC-MS/MS vs UHPLC-HRMS: Evaluation of the suitability for multitarget analysis

In order to assess their suitability for multitarget analysis, the linearity, LOQ$_{INS}$, and detection matrix effect of UHPLC-MS/MS and UHPLC-HRMS were compared. Both methodologies were evaluated using the optimum extraction and clean-up protocol outlined in *section 2.3*.

3.3.1. Linearity, repeatability and LOQ$_{INS}$

Both analysis techniques, UHPLC-MS/MS and UHPLC-HRMS, showed adequate linearity over 0.25–200 μg·kg^{-1} concentration range with determination coefficients (r^2) higher than 0.97 (see Table S3).

Regarding instrumental and intermediate repeatability, overall, more repeatable values were get using UHPLC-MS/MS. Concretely, RSD < 20 % were obtained using UHPLC-MS/MS for all compounds at low concentration levels, except for erythromycin (RSD values of 30–47 % in the concentration range of 0.25–1.00 μg·kg^{-1}). In the case of UHPLC-HRMS analysis, those low RSD values (i.e., < 20 %) were only obtained for some FQs and TCs at 1.00 μg·kg^{-1} and above concentrations.

Regarding LOQ$_{INS}$ values, using UHPLC-MS/MS provided LOQ$_{INS}$ in the range of 0.2–1.4 μg·kg^{-1}, similar to the ones reported by Tadić et al. (Tadić et al., 2019) by UHPLC-MS/MS analysis (0.4–1.7 μg·kg^{-1}) and lower to those get by UHPLC-HRMS in this work, especially in the case of tetracyclines and macrolides, irrespective of the use of discovery (LOQ$_{INS}$ 0.2–20 μg·kg^{-1}) or confirmation (LOQ$_{INS}$ between 0.2 and 59 μg·kg^{-1}) acquisition modes. For example, erythromycin could not be quantified due to the lack of MS2 when the analysis was performed using UHPLC-HRMS at the discovery acquisition mode; and therefore, no LOQ$_{INS}$ could be given for this compound under those conditions. Thus, when target analysis of AMs at low concentration levels is required, UHPLC-MS/MS using the DMRM acquisition mode provides better results in comparison to UHPLC-HRMS.

3.3.2. Matrix effect at the detection

Regardless of the instrumental technique used, matrix components can disrupt the ionisation of target AMs at the ionisation source, leading to decreased sensitivity and reproducibility, manifested as signal suppression or enhancement (Van De Steene & Lambert, 2008; Zhou et al., 2017). The detection matrix effect values obtained for all the evaluated matrices are depicted in Fig. 1 (UHPLC-MS/MS), Fig. 2a and 2b (UHPLC-HRMS in confirmation (a) and discovery (b) modes).

In the case of UHPLC-MS/MS, signal enhancement was mainly observed for onion, carrot and tomato matrices, with this effect being particularly noticeable for some TCs and SAs, which showed a ME % > 30 % (i.e., sulfamethazine 42–96 %, oxytetracycline 61–110 % and doxycycline 89–147 % among others). In the case of lettuce, a negative matrix effect predominates, with ofloxacin (-41 %) and roxithromycin (-46 %) standing out. These results are in agreement with those reported in the literature for FQs, SAs and trimethoprim analysis in lettuce, as they observed a matrix effect of less than 15 % with the exception of ofloxacin, and also enrofloxacin in this work (Tadić et al., 2019). The results obtained by He et al. in cabbage are similar to the ones reported
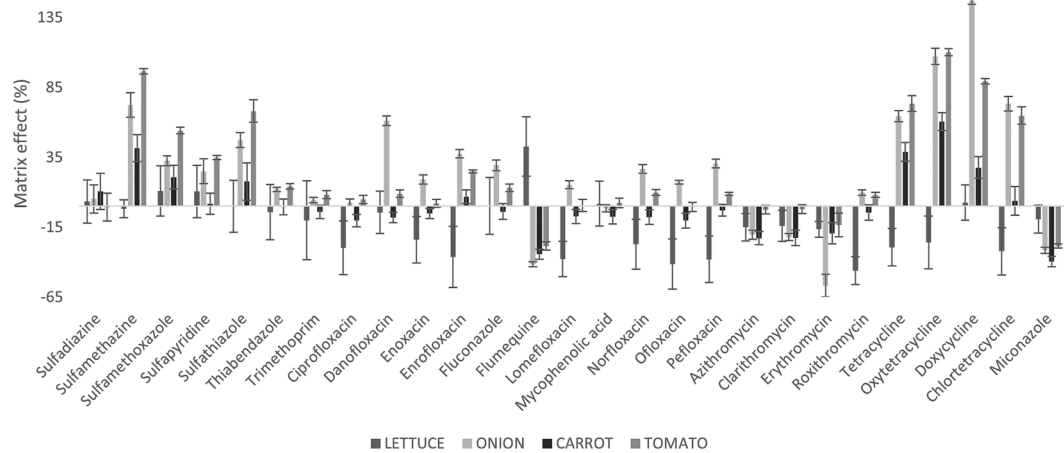
Fig. 1. Matrix effect at the detection by UHPLC-MS/MS analysis for the target AMs in each of the studied matrices (n = 3, uncertainty expressed as RSD %).
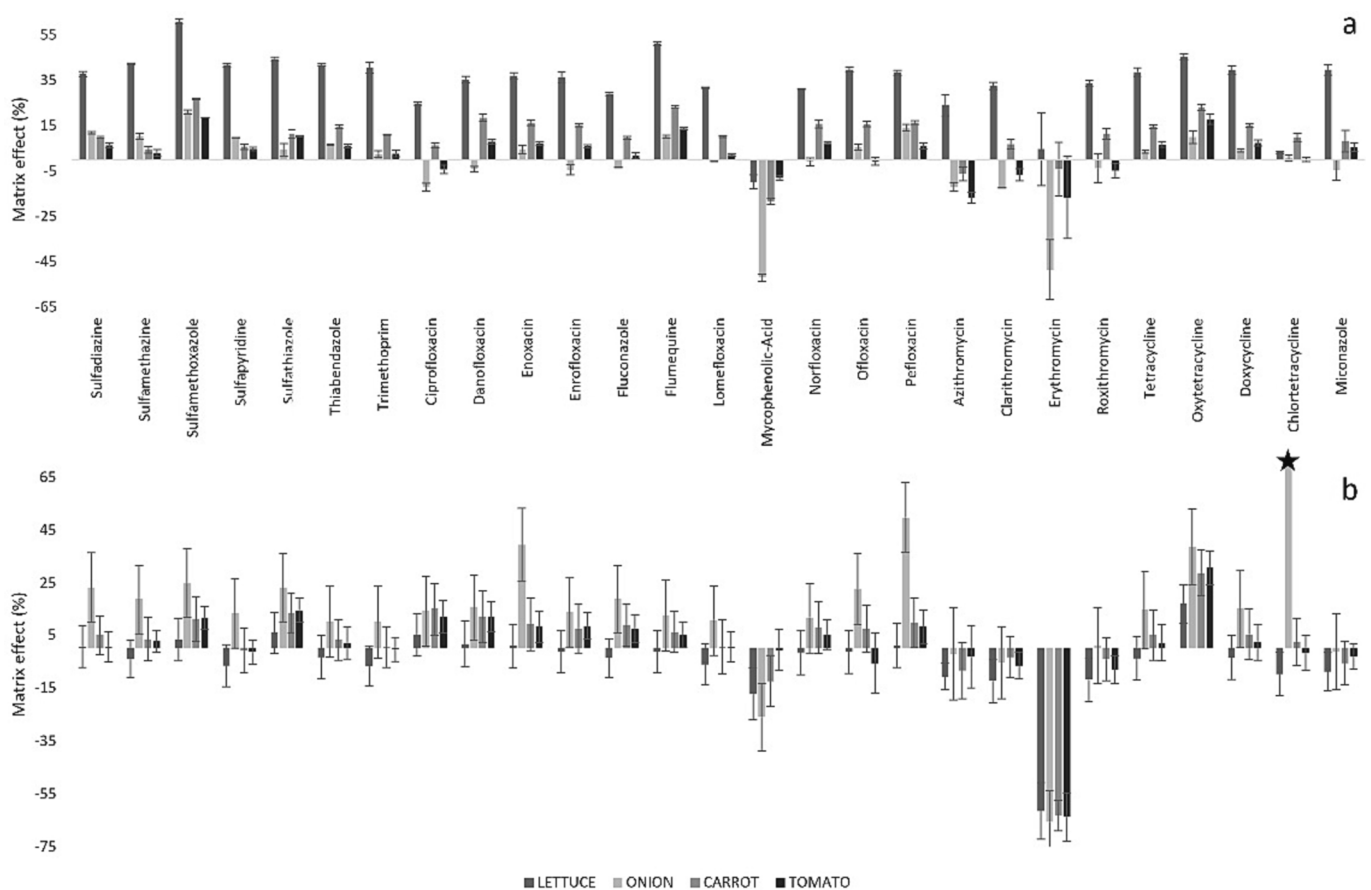

Fig. 2. Matrix effect at the detection by UHPLC-HRMS analysis for the target AMs in each of the studied matrices: in confirmation (a) and discovery (b) modes (n = 3, uncertainty expressed as RSD %).

in this work for FQs, clarithromycin and roxithromycin in lettuce (He et al., 2018).

A similar phenomenon was observed in UHPLC-HRMS confirmation mode, where a positive matrix effect was observed for compounds detected in all matrices, except lettuce, but being less pronounced than using UHPLC-MS/MS, as ME % < 30 % were obtained with the exception of chlortetracycline (136 %) and enoxacin (39 %) in onion. However, in this acquisition mode a significant negative matrix effect for erythromycin ((−61)-(−64) %) was observed in all the vegetable matrices. As for the UHPLC-HRMS discovery mode, an overall positive

matrix effect was observed for targets detected in all vegetable matrices, but less pronounced than using UHPLC-MS/MS (ME % < 30 % except for lettuce (3–61 %)). As exception, a signal suppression was observed for erythromycin ((-4)-(−49) %) and mycophenolic acid ((-8)-(−52) %).

Although UHPLC-HRMS provides a higher selectivity in the analysis, showing a lower matrix effect in the detection compared to UHPLC-MS/ MS, the significantly lower LOQ_{INS} obtained using the latter technique demonstrated its higher sensitivity to quantify AMs at lower concentration levels. Moreover, the matrix effect associated with UHPLC-MS/ MS can be corrected by different strategies, such as the use of

deuterated analogues as surrogates, which has been applied in this work (see **section 3.3.1**). These results are in line with those reported in the literature. For instance, the comparison of UHPLC-QqQ and UHPLC-qTOF to determine veterinary AMs in animal tissues carried out by Anumol et al. showed that QqQ provides low limits of quantification as long as the detection matrix effect was not too pronounced whereas q-TOF was a prime strategy to broaden the analytical coverage by the monitoring untargeted AMs (Anumol et al., 2017). In another study, Giusepponi et al. compared MS/MS and HRMS performance characteristics for the analysis of sixty AMs in bovine muscle and milk. A higher selectivity was attributed to HRMS in comparison to MS/MS, however, the use of HRMS affected the sensitivity of the method for milk analysis due to the massive presence of interfering substances (Giusepponi et al., 2019). Therefore, considering all of the above, it was decided to use UHPLC-MS/MS for multitarget analysis and target compounds quantification and UHPLC-HRMS to latter extend the method to the identification of unknown compounds or TPs.

3.4. Multitarget d-SPE-UHPLC-MS/MS analysis

3.4.1. Trueness and precision

Vegetable matrices (i.e., lettuce, onion, carrot and tomato) were spiked at 5 µg·kg^{-1}, 25 µg·kg^{-1} and 50 µg·kg^{-1} with all the AMs prior to the sample treatment explained in **section 1.2.** The absolute recoveries of the multitarget method in each of the studied vegetables are gathered in **Figure S5**. Trueness was verified by the determination of analytes' apparent recoveries using both, surrogate correction and matrix-matched calibration approaches, whereas repeatability was calculated in terms of RSD (%) (see the results in **Tables S4-S7**).

Absolute recoveries ranged from 45 to 187 %, 41–212 %, 52–187 % and 30–129 % in compounds analysed in lettuce, onion, tomato, and carrot, respectively. Trueness determined by surrogate correction in the range of 35–178 % for targets measured in lettuce, 34–188 % in onion, 40–141 % in carrot and 39–169 % in tomato. Compared to the values reported by Tadić et al. at concentration levels of 10 µg·kg^{-1} and 100 µg·kg^{-1}, in this work accurate results were obtained for sulfadiazine and sulfathiazole at low concentration in lettuce, as well as for enrofloxacin at low and high levels in lettuce and tomato. However, they retrieved better results for trimethoprim in lettuce and sulfadiazine in tomato (Tadić et al., 2019). Similar results were also reported by He et al. in cabbage at 5 µg·kg^{-1} and 50 µg·kg^{-1} for SAs, TCs, FQs and MCs (He et al., 2018).

Using matrix-matched calibration approach more truthful results were obtained according to the Commission Implementing Regulation (EU) 2021/808 of 22 March 2021 (i.e., 70–120 % for 1–10 µg·kg^{-1} concentrations, 80–120 % for concentrations > 10 µg·kg^{-1} and precision, expressed as RSD %, ≤ 30 %) (Commission Implementing Regulation (EU) 2021/808 of 22 March 2021 on the Performance of Analytical Methods for Residues of Pharmacologically Active Substances Used in Food-Producing Animals and on the Interpretation of Results as Well as on the Methods to Be Used for Sampling and Repealing Decisions 2002/657/EC and 98/179/EC (Text with EEA Relevance), 2021). These recoveries ranged from 67 to 134 % for targets determined in lettuce (with the exception of sulfathiazole at 5 µg·kg^{-1}), 83–121 % in onion (with the exception of erythromycin at 5 µg·kg^{-1}), 84–132 % in carrot (with the exception of erythromycin at 5 µg·kg^{-1}), and 75–136 % in tomato.

Chuang et al. (Chuang et al., 2015) reported apparent recovery values calculated with matrix-matched calibration for sulfadiazine, sulfamethoxazole, oxytetracycline and trimethoprim in lettuce at 200 µg·kg^{-1} concentration level (74 %, 74 %, 72 % and 82 %, respectively) which are in concordance with the ones determined in this work at the highest validation level, 50 µg·kg^{-1} (86 %, 92 %, 91 % and 101 % for sulfadiazine, sulfamethoxazole, oxytetracycline and trimethoprim, respectively).

Regardless of the strategy used for the calculation of apparent

Table 1
Compounds annotated at the vegetables samples at levels 1–5 using suspect screening by UHPLC-HRMS.

Feature	Accurate mass	Exact mass error (ppm)	Formula	Name	RT (min)	Confidence level	Organic Vegetables				Non-Organic Vegetables			
							Lettuce	Onion	Carrot	Tomato	Lettuce	Onion	Carrot	Tomato
1	280.16719	−0.96	$C_{16}H_{24}O_4$	Brefeldin A	12.724	2b								✓
2	475.30643	−3.03	$C_{21}H_{41}N_5O_7$	Netilmicin	7.741	2a							✓	✓
3	145.05278	0.10	C_9H_7NO	Indole-4-carboxaldehyde	7.071	2a	✓		✓		✓	✓	✓	✓
4	150.10445	−0.11	$C_{10}H_{14}O$	Carvone	13.177	2a		✓	✓		✓	✓	✓	
5	306.11003	−0.99	$C_{16}H_{18}O_6$	TP of mycophenolic acid	13.793	2b								

recoveries, RSD values lower than 30 % were obtained for all of the analytes and matrices, indicating good precision and in compliance with the regulation.

3.4.2. LOQ_{PROC}

LOQ_{PROC} obtained in this work are gathered in **Table S8**. All in all, this work offered better LOQ_{PROC} with a range between 0.1 and 2.8 $\mu g \cdot kg^{-1}$ compared to those obtained by He et al, (2.0–5.0 $\mu g \cdot kg^{-1}$) (He et al., 2018) and Yu et al. (1.1–5.8 $\mu g \cdot kg^{-1}$) (Yu et al., 2018).

3.5. Suspect screening using d-SPE-UHPLC-qOrbitrap

Discovery acquisition mode was preferably selected for the identification of unknown compounds, as it is difficult to gather the necessary information from unknown compounds to perform an analysis in confirmation acquisition mode (i.e. molecular formula, exact mass and retention time). LOI_{INS} and LOI_{PROC} were calculated in discovery mode for the twenty-seven AMs and are included in **Table S9**.

LOI_{INS} ranged from 0.2 $\mu g \cdot kg^{-1}$ to 8.9 $\mu g \cdot kg^{-1}$ with the exception of doxycycline, chlortetracycline and azithromycin for which values of 18 $\mu g \cdot kg^{-1}$, 36 $\mu g \cdot kg^{-1}$ and 38 $\mu g \cdot kg^{-1}$, respectively, were obtained. In the presence of matrix, the lowest LOI_{PROC} were observed for tomato (i.e., 1.6–13.0 $\mu g \cdot kg^{-1}$ except for chlortetracycline and azithromycin), for which the lowest LOQ_{PROC} were also calculated for some TCs, MCs and FQs, especially compared to lettuce, where the highest LOI_{INS} were estimated. This may be due to the chlorophyll content of lettuce (Costache, M. A. et al., 2012), which could interfere with the sample treatment process leading to a loss of sensitivity. Comparing the AM families, the lowest LOIs, both LOI_{INS} and LOI_{PROC}, were estimated for SAs followed by FQs, while MCs and TCs showed the highest values. Neither LOI_{INS} nor LOI_{PROC} could be calculated for erythromycin due to the lack of fragmentation spectra. Erythromycin was unsuccessfully ionised in the UHPLC-HRMS in accordance with the poor results obtained also for the standards and therefore, when acquired in ddMS2-discovery mode, could not be selected for further fragmentation in the Orbitrap.

Comparing with the literature, González-Gaya et al. obtained LOI_{INS} ranging from 2.4 to 263 $\mu g \cdot kg^{-1}$ (González-Gaya et al., 2021) for different antibiotics in water using the qOrbitrap instrument, being higher than those obtained in this work. However, no work has been found reporting LOI_{PROC} in vegetables other than those validated here, although as seen in this work for the different types of vegetables analysed, LOI_{PROC} can vary greatly between matrices. These results further increase the need for more work applying suspect screening analysis to a variety of environmental samples.

4. APPLICATION TO REAL SAMPLES

The validated methods to determine AMs and TPs were used in two case studies in order to evaluate their applicability in environmental concerns: (i) occurrence and degradation of AMs in vegetable samples, and (ii) uptake and degradation of AMs in vegetable samples cultivated in contaminated soils.

4.1. Evaluation of AMs and their TPs presence in vegetables from the Basque Country

We conducted a thorough analysis of thirty samples of lettuce, onion, carrot, and tomato obtained from local markets in the Basque Country. The aim was to quantify AMs using UHPLC-MS/MS and to screen for any potential suspect using UHPLC-HRMS. The target analysis in carrots from non-organic and organic agriculture by UHPLC-MS/MS allowed the detection of two AMs, sulfadiazine and mycophenolic acid, below the LOQ_{PROC}.

Regarding suspect screening, by applying the filters previously mentioned in **section 2.5.2**, the number of candidates was reduced from the initial 13,798 to 77. Following this, manual peak picking was carried out, resulting in 35 features that met the constraints for peak shape. After careful consideration of multiple quality criteria, including a MS2 match of over 70 % based on the mzCloud database, in-silico fragmentation by Mass Frontier, and/or entries in ChemSpider, as well as a matching retention time predicted by the RTI model, we annotated 5 key features. Table 1 includes the features annotated at levels 1–5 according to the Schymanski scale. Netilmicin (semisynthetic aminoglycoside antibiotic) was identified at level 2a and Brefeldin A (antiviral drug) at level 2b. Netilmicin was found in three carrots, two from organic markets and one from non-organic markets, while Brefeldin A was found in fresh tomatoes bought in non-organic markets. The presence of bacteria resistant to netilmicin in vegetables have been reported (Schwaiger et al., 2011), which might indicate the previous presence of this compound in those vegetable matrices or in their near environment, such as sediments or compost used as fertilisers (Quaik et al., 2020). Indole-4-carboxaldehyde (used for pharmaceutical synthesis), identified at level 2a, was found in lettuces and carrots from organic and non-organic markets as well as in two tomatoes and one onion from non-organic markets. Similarly, the presence of the antifungal agent carvone, identified at level 2a, was detected in lettuces and carrots from organic and non-organic markets as well as in two tomatoes and one onion from non-organic markets. Carvone has been reported to be used against tomato root-knot caused by *Meloidogyne incognita*, which could explained the presence of this compound in such matrix (Elsharkawy et al., 2022).
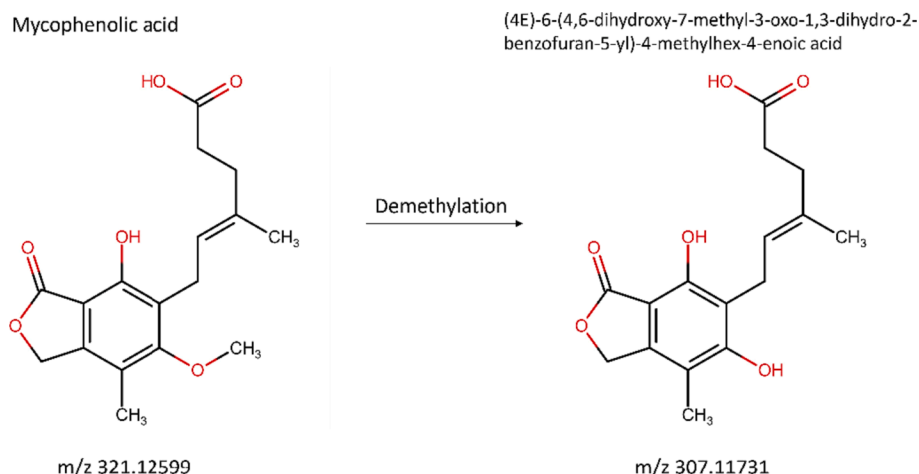

Fig. 3. Chemical structures of mycophenolic acid and a demethylated TP of mycophenolic acid.

Table 2

Exposition experiment concentrations in soils and the corresponding lettuces, together with the soil adsorption coefficient (K_{oc}) and half-life times of each of the studied antimicrobials.

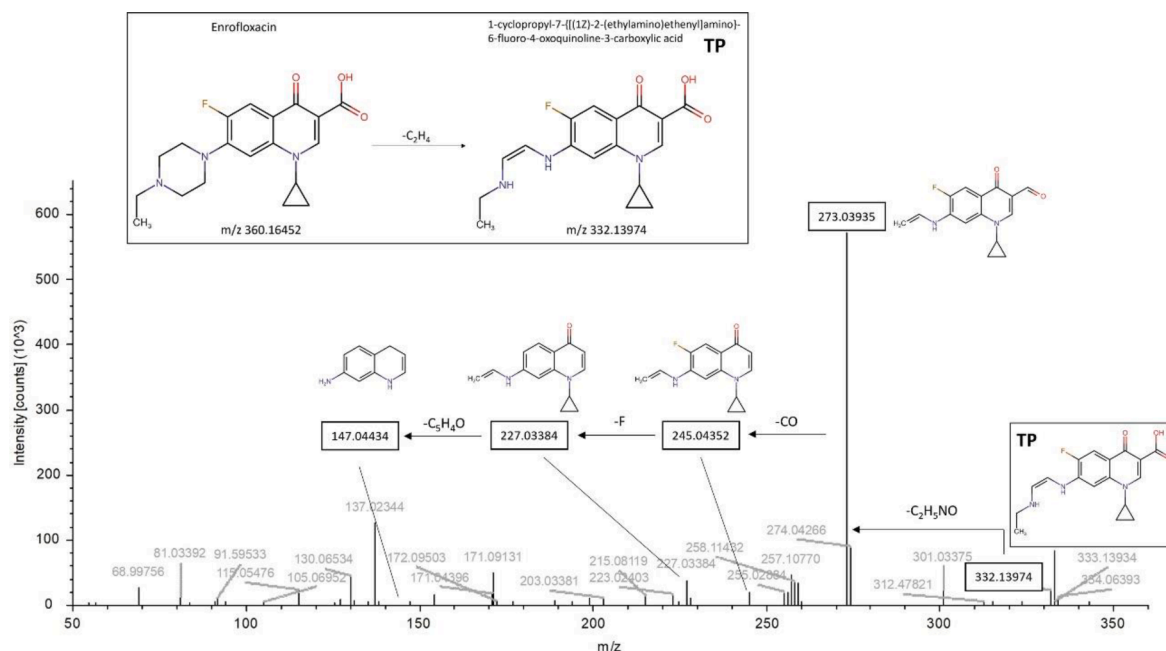|	[a]Soil adsorption coeff. (Koc) L·kg^{-1}	[a]Half-life (days)	Soil (µg·kg^{-1})		Lettuce (µg·kg^{-1})	
			1st week	3rd week	1st week	3rd week
Sulfamethazine	143	3.4	50 ± 3	8 ± 1	0.3 ± 0.1	0.2 ± 0.1
Enrofloxacin	481	3.4	183 ± 7	82 ± 3	9.1 ± 0.1	1.3 ± 0.1
Trimethoprim	115	4.2	124 ± 9	43 ± 3	2.9 ± 0.4	0.4 ± 0.1
Chlortetracycline	31	148	593 ± 12	303 ± 6	15 ± 3	2.0 ± 0.5

[a] (US EPA, 2023).

Fig. 4. Chemical structures of enrofloxacin and the identified TP and the structures proposed for the ion fragments in the MS2 of the TP.

Finally, a TP derived from the demethylation of mycophenolic acid (see Fig. 3), (4E)-6-(4,6-dihydroxy-7-methyl-3-oxo-1,3-dihydro-2-benzofuran-5-yl)-4-methylhex-4-enoic acid, was tentatively identified at level 2b and found in carrots from organic and non-organic agriculture, consistent with the presence of its predecessor mycophenolic acid in the same samples. There is limited information on the occurrence of mycophenolic acid in vegetables. However, it has been reported to be synthesised by *Byssochlamys nivea*, which is a fungi species responsible for the spoilage of vegetables and fruits. Moreover, as it can survive heat treatments used for food processing and can grow during storage at room temperature (Puel et al., 2005), it is not unusual to detect its presence even in the vegetables. See the chromatograms for some of the annotated compounds in **Figures S6-S8**.

4.2. Study of the transfer of ams to lettuces grown in contaminated soils

The quantified concentrations for each AM in both matrices, soil and the corresponding lettuce, can be seen in Table 2.

The results indicate a significant decline in the presence of AMs in the soil during the initial week after spiking. Notably, sulfamethazine experienced an impressive degradation rate of 95 %, closely followed by trimethoprim which showed a substantial degradation of 88 % within the same timeframe. Over time, the degradation continued, resulting in significantly lower detection of AMs in the soils during the third week. Nonetheless, there was an observed transfer of AMs from the soil to the lettuce. Similar to the soil, a higher initial concentration was detected compared to the concentration observed in the third week. This suggests that degradation of AMs also occurs in the plant.

The behaviour of each AM in soil and its mobility towards the plant can be better understood considering the half-life and adsorption coefficient (K_{oc}) of AMs in soil (values obtained from the Environmental Protection Agency (EPA) (US EPA, 2023) are gathered in Table 2). For example, considering the degradation time of chlortetracycline, it makes sense that it is the compound detected in the highest concentration in soil and, considering its K_{oc}, it is the one most transferred to lettuce. As for sulfamethazine, its K_{oc} and degradation time also agree with the results, as it is the compound detected at the lowest concentration in the soil and its transfer to lettuce is minimal.

The samples were also analysed using suspect screening approach and a TP of enrofloxacin, 1-cyclopropyl-7-{[(1Z)-2-(ethylamino) ethenyl]amino}-6-fluoro-4-oxoquinoline-3-carboxylic acid, was detected in the exposed lettuce samples. The chemical structure of the TP, the obtained MS2 and proposed structures for the fragment ions are gathered in Fig. 4. Sulfamethazine was also identified at 2a level in the spiked soils and the lettuces grown in there.

5. Conclusions

This work successfully validated an analytical method capable of accurately and simultaneously determining a wide range of AMs at trace concentration levels in several vegetable samples by multitarget analysis. In addition, it has also demonstrated its potential to identify unknown AMs and their TPs in vegetable matrices. The lower LOQ_{INS} obtained and the possibility of controlling the matrix effect at the

detection with the use of surrogates, made us to preferably consider UHPLC-MS/MS technique to perform the multitarget analysis. However, this work highlights the importance of working with both low- and high-resolution analysis techniques to perform a comprehensive AM contamination monitoring, as the use of UHPLC-HRMS has allowed to detect the formation and presence of specific TPs of AMs in vegetables, which is not feasible with the target analysis.

The methods applied in several case studies have yielded remarkable results. On the one hand, the observations found in uptake experiments showed that AMs transference occur from contaminated soils to lettuce. Although this last conclusion, the occurrence of AMs studied in vegetable samples gathered in several commercial points in the Basque Country was negligible and only sulfadiazine and mycophenolic acid were detected in carrots from non-organic and organic agriculture below LOQ_{PROC}. Therefore, vegetables do not seem to be an important source of AM contamination in the Basque Country. However, the discovery of AMs' by-products highlights the need for further research to identify the potential risks of these compounds and to establish the necessary regulations to control them. Although there are currently some thresholds for the presence of AMs in environmental samples, there are no regulations regarding their TPs. Thus, efforts should be focused on the investigation and identification ofTPs due to the varying environmental conditions that can lead to various reactions and the formation of different products.

CRediT authorship contribution statement

I. Vergara-Luis: Investigation, Data curation, Validation, Formal analysis, Methodology, Writing – original draft, Visualization. **M. Jin:** Investigation, Data curation. **J.C. Baez-Millán:** Investigation, Validation. **B. González-Gaya:** Investigation, Supervision. **I. Ijurco:** Sample acquisition. **M. Lacuesta:** Sample acquisition, Funding acquisition. **M. Olivares:** Methodology, Conceptualization, Formal analysis, Funding acquisition, Writing – review & editing. **A. Prieto:** Supervision, Methodology, Conceptualization, Formal analysis, Writing – review & editing, Funding acquisition, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.138643.

References

Anumol, T., Lehotay, S. J., Stevens, J., & Zweigenbaum, J. (2017). Comparison of veterinary drug residue results in animal tissues by ultrahigh-performance liquid chromatography coupled to triple quadrupole or quadrupole–time-of-flight tandem mass spectrometry after different sample preparation methods, including use of a commercial lipid removal product. *Analytical and Bioanalytical Chemistry, 409*(10), 2639–2653. https://doi.org/10.1007/s00216-017-0208-y

Barron, L., Tobin, J., & Paull, B. (2008). Multi-residue determination of pharmaceuticals in sludge and sludge enriched soils using pressurized liquid extraction, solid phase extraction and liquid chromatography with tandem mass spectrometry. *Journal of Environmental Monitoring, 10*(3), 353. https://doi.org/10.1039/b717453e

Burhenne, J., Ludwig, M., & Spiteller, M. (1999). Polar photodegradation products of quinolones determined by HPLC/MS/MS. *Chemosphere, 38*(6), 1279–1286. https://doi.org/10.1016/S0045-6535(98)00525-6

Castellani, F., Ricci, M., Colagrande, M. N., Scortichini, G., & Saluti, G. (2023). Development and Validation of a Confirmatory Method for the Determination of 12 Coccidiostat Residues in Eggs and Muscle by Means of Liquid Chromatography Coupled to Hybrid High Resolution Mass Spectrometry. *Separations, 10*(3), 202. Scopus. https://doi.org/10.3390/separations10030202.

Chuang, Y.-H., Zhang, Y., Zhang, W., Boyd, S. A., & Li, H. (2015). Comparison of accelerated solvent extraction and quick, easy, cheap, effective, rugged and safe method for extraction and determination of pharmaceuticals in vegetables. *Journal of Chromatography A, 1404*, 1–9. https://doi.org/10.1016/j.chroma.2015.05.022

2002/657/EC: Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (Text with EEA relevance) (notified under document number C(2002) 3044), 221 OJ L (2002). http://data.europa.eu/eli/dec/2002/657/oj/eng.

Commission Implementing Regulation (EU) 2021/808 of 22 March 2021 on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results as well as on the methods to be used for sampling and repealing Decisions 2002/657/EC and 98/179/EC (Text with EEA relevance), 180 OJ L (2021). http://data.europa.eu/eli/reg_impl/2021/808/oj/eng.

Costache, M. A., Campeanu, G., & Neata, G. (2012). *Studies concerning the extraction of chlorophyll and total carotenoids from vegetables. 8. 17*(5), 7702-7708.

Cycoń, M., Mrozik, A., & Piotrowska-Seget, Z. (2019). Antibiotics in the Soil Environment—Degradation and Their Impact on Microbial Activity and Diversity. *Frontiers in Microbiology, 10*, 338. https://doi.org/10.3389/fmicb.2019.00338

da Silva, J. J., da Silva, B. F., Stradiotto, N. R., Petrovic, M., Gago-Ferrero, P., & Gros, M. (2020). Pressurized Liquid Extraction (PLE) and QuEChERS evaluation for the analysis of antibiotics in agricultural soils. *MethodsX, 7*, Article 101171. https://doi.org/10.1016/j.mex.2020.101171

Dasenaki, M. E., & Thomaidis, N. S. (2015). Multi-residue determination of 115 veterinary drugs and pharmaceutical residues in milk powder, butter, fish tissue and eggs using liquid chromatography–tandem mass spectrometry. *Analytica Chimica Acta, 880*, 103–121. https://doi.org/10.1016/j.aca.2015.04.013

Elsharkawy, M. M., Al-Askar, A. A., Behiry, S. I., Abdelkhalek, A., Saleem, M. H., Kamran, M., & Derbalah, A. (2022). Resistance induction and nematicidal activity of certain monoterpenes against tomato root-knot caused by Meloidogyne incognita. *Frontiers in Plant Science, 13*, Article 982414. https://doi.org/10.3389/fpls.2022.982414

García, J., García-Galán, M. J., Day, J. W., Boopathy, R., White, J. R., Wallace, S., & Hunter, R. G. (2020). A review of emerging organic contaminants (EOCs), antibiotic resistant bacteria (ARB), and antibiotic resistance genes (ARGs) in the environment: Increasing removal with wetlands and reducing environmental impacts. *Bioresource Technology, 307*, Article 123228. https://doi.org/10.1016/j.biortech.2020.123228

Giusepponi, D., Paoletti, F., Barola, C., Moretti, S., Saluti, G., Ianni, F., Sardella, R., & Galarini, R. (2019). Transfer of a multiclass method for over 60 antibiotics in food from high resolution to low resolution mass spectrometry. *Molecules, 24*(16), 2935. Scopus. https://doi.org/10.3390/molecules24162935.

González-Gaya, B., Lopez-Herguedas, N., Bilbao, D., Mijangos, L., Iker, A. M., Etxebarria, N., Irazola, M., Prieto, A., Olivares, M., & Zuloaga, O. (2021). Suspect and non-target screening: The last frontier in environmental analysis. *Analytical Methods, 13*(16), 1876–1904. https://doi.org/10.1039/D1AY00111F

Hang, L., Zhao, Y., Liu, C., Yu, Y., He, Y., Xu, J., & Lu, Z. (2021). Determine Multiple Classes of Veterinary Antibiotics in Soil: Comparing Dispersive and Solid-Phase Extraction for Sample Cleanup. *Chromatographia, 84*(9), 833–844. https://doi.org/10.1007/s10337-021-04064-5

He, Z., Wang, Y., Xu, Y., & Liu, X. (2018). Determination of Antibiotics in Vegetables Using QuEChERS-Based Method and Liquid Chromatography-Quadrupole Linear Ion Trap Mass Spectrometry. *Food Analytical Methods, 11*(10), 2857–2864. https://doi.org/10.1007/s12161-018-1252-8

Jadeja, N. B., & Worrich, A. (2022). From gut to mud: Dissemination of antimicrobial resistance between animal and agricultural niches. *Environmental Microbiology, 24*(8), 3290–3306. https://doi.org/10.1111/1462-2920.15927

Jongedijk, E., Fifeik, M., Arrizabalaga-Larrañaga, A., Polzer, J., Blokland, M., & Sterk, S. (2023). Use of high-resolution mass spectrometry for veterinary drug multi-residue

analysis. *Food Control*, *145*, 109488. Scopus. https://doi.org/10.1016/j. foodcont.2022.109488.

Kang, D. H., Gupta, S., Rosen, C., Fritz, V., Singh, A., Chander, Y., Murray, H., & Rohwer, C. (2013). Antibiotic Uptake by Vegetable Crops from Manure-Applied Soils. *Journal of Agricultural and Food Chemistry, 61*(42), 9992–10001. https://doi. org/10.1021/jf404045m

Li, W., Shi, Y., Gao, L., Liu, J., & Cai, Y. (2013). Occurrence, distribution and potential affecting factors of antibiotics in sewage sludge of wastewater treatment plants in China. *Science of The Total Environment, 445–446*, 306–313. https://doi.org/ 10.1016/j.scitotenv.2012.12.050

Meng, M., He, Z., Xu, Y., Wang, L., Peng, Y., & Liu, X. (2017). Simultaneous extraction and determination of antibiotics in soils using a method based on quick, easy, cheap, effective, rugged, and safe extraction and liquid chromatography with tandem mass spectrometry. *Journal of Separation Science, 40*(16), 3214–3220. https://doi.org/ 10.1002/jssc.201700128

Mijangos, L., Ziarrusta, H., Zabaleta, I., Usobiaga, A., Olivares, M., Zuloaga, O., Etxebarria, N., & Prieto, A. (2019). Multiresidue analytical method for the determination of 41 multiclass organic pollutants in mussel and fish tissues and biofluids by liquid chromatography coupled to tandem mass spectrometry. *Analytical and Bioanalytical Chemistry, 411*(2), 493–506. https://doi.org/10.1007/s00216-018-1474-z

Puel, O., Tadrist, S., Galtier, P., Oswald, I. P., & Delaforge, M. (2005). Byssochlamys nivea as a Source of Mycophenolic Acid. *Applied and Environmental Microbiology, 71* (1), 550–553. https://doi.org/10.1128/AEM.71.1.550-553.2005

Quaik, S., Embrandiri, A., Ravindran, B., Hossain, K., Al-Dhabi, N. A., Arasu, M. V., Ignacimuthu, S., & Ismail, N. (2020). Veterinary antibiotics in animal manure and manure laden soil: Scenario and challenges in Asian countries. *Journal of King Saud University - Science, 32*(2), 1300–1305. https://doi.org/10.1016/j.jksus.2019.11.015

Rodriguez-Aller, M., Gurny, R., Veuthey, J.-L., & Guillarme, D. (2013). Coupling ultra high-pressure liquid chromatography with mass spectrometry: Constraints and possible applications. *Journal of Chromatography A, 1292*, 2–18. https://doi.org/ 10.1016/j.chroma.2012.09.061

Ryder, E. J. (s. f.). *The New Salad Crop Revolution*. 408-412.

Schwaiger, K., Helmke, K., Hölzel, C. S., & Bauer, J. (2011). Antibiotic resistance in bacteria isolated from vegetables with regards to the marketing stage (farm vs. Supermarket). *International Journal of Food Microbiology, 148*(3), 191–196. https:// doi.org/10.1016/j.ijfoodmicro.2011.06.001

Schymanski, E. L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H. P., & Hollender, J. (2014). Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence. *Environmental Science & Technology, 48*(4), 2097–2098. https://doi.org/10.1021/es5002105

Sun, L., Jia, L., Xie, X., Xie, K., Wang, J., Liu, J., Cui, L., Zhang, G., Dai, G., & Wang, J. (2016). Quantitative analysis of amoxicillin, its major metabolites and ampicillin in eggs by liquid chromatography combined with electrospray ionization tandem mass

spectrometry. *Food Chemistry, 192*, 313–318. https://doi.org/10.1016/j. foodchem.2015.07.028

Sunyer-Caldú, A., & Diaz-Cruz, M. S. (2021). Development of a QuEChERS-based method for the analysis of pharmaceuticals and personal care products in lettuces grown in field-scale agricultural plots irrigated with reclaimed water. *Talanta, 230*, Article 122302. https://doi.org/10.1016/j.talanta.2021.122302

Tadić, Đ., Matamoros, V., & Bayona, J. M. (2019). Simultaneous determination of multiclass antibiotics and their metabolites in four types of field-grown vegetables. *Analytical and Bioanalytical Chemistry, 411*(20), 5209–5222. https://doi.org/ 10.1007/s00216-019-01895-y

US EPA, O. (2023, abril 11). *Computational Toxicology and Exposure Online Resources* [Collections and Lists]. https://www.epa.gov/comptox-tools.

Valcárcel, Y., González Alonso, S., Rodríguez-Gil, J. L., Gil, A., & Catalá, M. (2011). Detection of pharmaceutically active compounds in the rivers and tap water of the Madrid Region (Spain) and potential ecotoxicological risk. *Chemosphere, 84*(10), 1336–1348. https://doi.org/10.1016/j.chemosphere.2011.05.014

Van De Steene, J. C., & Lambert, W. E. (2008). Comparison of matrix effects in HPLC-MS/ MS and UPLC-MS/MS analysis of nine basic pharmaceuticals in surface waters. *Journal of the American Society for Mass Spectrometry, 19*(5), 713–718. https://doi. org/10.1016/j.jasms.2008.01.013

Varenina, I., Bilandžić, N., Luburić, Đ. B., Kolanović, B. S., & Varga, I. (2022). High resolution mass spectrometry method for the determination of 13 antibiotic groups in bovine, swine, poultry and fish meat: An effective screening and confirmation analysis approach for routine laboratories. *Food Control, 133*, 108576. Scopus. https://doi.org/10.1016/j.foodcont.2021.108576.

Vergara-Luis, I., Báez-Millán, J. C., Baciero, I., González-Gaya, B., Olivares, M., Zuloaga, O., & Prieto, A. (2023). Comparison of conventional and dispersive solid phase extraction clean-up approaches for the simultaneous analysis of tetracyclines and sulfonamides in a variety of fresh vegetables. *Talanta, 254*, Article 124192. https://doi.org/10.1016/j.talanta.2022.124192

Vergara-Luis, I., Bocayá, N., Irazola-Duñabeitia, M., Zuloaga, O., Lacuesta, M., Olivares, M., & Prieto, A. (2023). Multitarget and suspect screening of antimicrobials in soil and manure by means of QuEChERS — liquid chromatography tandem mass spectrometry. *Analytical and Bioanalytical Chemistry, 415*, 6291–6310. https://doi. org/10.1007/s00216-023-04905-2

Yu, X., Liu, H., Pu, C., Chen, J., Sun, Y., & Hu, L. (2018). Determination of multiple antibiotics in leafy vegetables using QuEChERS-UHPLC-MS/MS. *Journal of Separation Science, 41*(3), 713–722. https://doi.org/10.1002/jssc.201700798

Zalewska, M., Błażejewska, A., Czapko, A., & Popowska, M. (2021). Antibiotics and Antibiotic Resistance Genes in Animal Manure – Consequences of Its Application in Agriculture. *Frontiers in Microbiology, 12*, Article 610656. https://doi.org/10.3389/ fmicb.2021.610656

Zhou, W., Yang, S., & Wang, P. G. (2017). Matrix effects and application of matrix effect factor. *Bioanalysis, 9*(23), 1839–1844. https://doi.org/10.4155/bio-2017-0214