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Statement of novelty of the manuscript

Comparison of MRM^{HR} and SWATH acquisition modes for the quantitation of 48 wastewater-borne pollutants in lettuce leaves using a modified QuEChERS method

The presence of anthropogenic contaminants in crops for human consumption is a topic of great interest because their presence encompasses a potential risk for humans. However, there is a lack of analytical methods for detecting wastewater-borne pollutants in crops. One of the major efforts of recent years is to try to develop rapid, simple, robust and sensitive analytical methods for the analysis of wastewater-borne pollutants in plant tissues.

Here, first we evaluated the best mode using high resolution mass spectrometry (HRMS) of wastewater-borne pollutants in lettuce leaves irrigated with treated wastewater. Also quick and simple QuEChERS-based method was developed for the extraction of these pollutants in field samples. The developed method will improve the understanding on environmental exposure of wastewater-borne pollutants in crops.

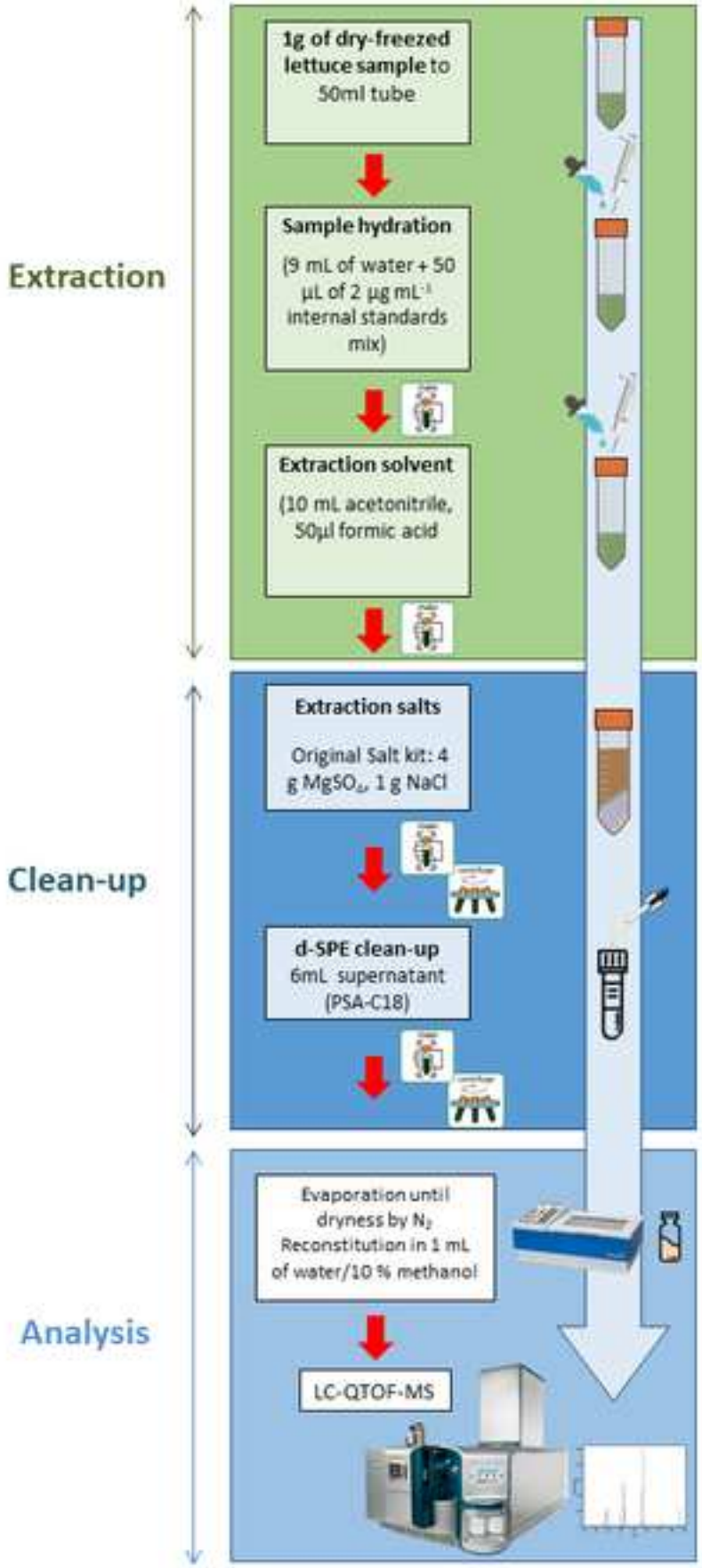
Highlights

Comparison of high resolution multiple reaction monitoring and SWATH acquisition MS modes

Development of a modified QuEChERS-based method for the determination of 48 PhACs in lettuce

Efficient analyte recoveries and low matrix effects using two-step cleanup

14 out of 48 studied compounds were detected in field samples



Comparison of MRM^{HR} and SWATH acquisition modes for the quantitation of 48 wastewater-borne pollutants in lettuce leaves using a modified QuEChERS method

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ABSTRACT

Screening of a large number of chemicals of emerging concern is highly desirable for the control of crops irrigated with reclaimed water since it is considered an alternative water source of great value. This study describes a high resolution mass spectrometry approach for developing methods for quantification in lettuce leaves of 48 different wastewater-borne pollutants (including analgesics and anti-inflammatories, anti-hypertensives, antifungal agents, lipid regulators, psychiatric drugs and stimulants, β-blockers, antibiotics, antimycotics, and sweeteners) frequently found in water resources. In this respect, a simple and fast QuEChERS-based method for the determination of contaminants in lettuce has been developed. During extraction, the use of formic acid was adopted to further improve the results of some problematic compounds (e.g., fenofibrate, furosemide, metronidazole, oxcarbazepine, sulfanilamide). High resolution multiple reaction monitoring (MRM^{HR}) and SWATH acquisition were compared in term of accuracy, repeatability, sensitivity, linearity and matrix effect. Both methods provided similar recoveries between 80 and 120% in lettuce leaves, although sulfanilamide, ciprofloxacin, and sulfamethazine presenting values of 26.8, 27.8, and 28.4% in MRM^{HR} and 25, 33.9, and 35% in SWATH, respectively. The effectiveness of a two-step cleanup on analyte recovery was also assessed and matrix effects were also taken into consideration during the method validation. The developed method allows the simultaneous quantitative analysis of 48 compounds (drug residues and metabolites) in lettuce leaves irrigated with treated wastewater for human consumption. Application of the present method to lettuce crops growth in controlled conditions showed the presence of 14 out 48 studied compounds with concentrations ranging from 2.9 ng g^{-1} (metoprolol) to 196.3 ng g^{-1} (citalopram). Drug residues such as sulfamethazine (33.2 ng g^{-1}), and carbamazepine (6.0 ng g^{-1}), and its metabolite carbamazepine epoxide (18.1 ng g^{-1}), frequently found in wastewater effluents, were also detected.

1. Introduction:

In communities where water is a limited commodity, traditional water resources such as surface and ground water cannot meet their demands. Therefore, to address present and future water shortages, alternative water sources are considered. The use of reclaimed water is of a great interest as a response to the high water demand in urban and rural areas, and, in fact this practice is already well established in agriculture which accounts for about 70% of freshwater consumption. However, reclaimed wastewater can contain salts, inorganic nitrogen and pathogens, heavy metals and organic contaminants such as pharmaceuticals which can present a potential risk not only to soil and the groundwater underneath but particularly to the crops. When pollutants are taken up into these plants during the growth phase bur are not eliminated by the time of harvest, they enter the food chain ultimately leading to undesired exposure of humans and animals to inherently bioactive substances. Consequently, there is growing concern about the human health impact of crops irrigated with reused water.

Lettuce is one of the fresh crops most consumed raw around the world [1] and as a leafy vegetable has a very high ability to take up pharmaceuticals in its edible tissues [2]. However, few studies have evaluated the presence of wastewater-borne in lettuce because of the lack of suitable analytical methods [1]. The development of multi-analyte extraction methods for the determination of trace levels of wastewater-borne in lettuce is challenging for two major reasons: on the one hand, drugs differ widely in their structures and consequently in their physicochemical properties, and thus behave differently in extraction and clean-up processes. On the other hand, plant tissues are of complex composition containing numerous endogenous components, such as pigments, fat, cellulose and wax, which are prone to interfere with the sample extraction and subsequent measurement of the analytes, if not removed during sample treatment [3-7].

In recent years, several analytical methods have been developed to extract wastewater-borne pollutants from plant tissues using traditional approaches such as solid-liquid extraction [8, 9], accelerated solvent extraction [7], and ultrasound extraction [1, 5, 10-13]. However, in order to assess the food quality and safety with respect to the presence of microcontaminants, a quick, selective, and sensitive analytical protocol is needed for its quantification in

harvested vegetables. For an innovative, rapid, simple, robust and sensitive method only few publications proposed the use of a quick, easy, cheap, effective, rugged and safe (QuEChERS)-based method for the determination of pharmaceuticals in lettuce or other vegetable commodities [7, 9, 14-18]. Chuang et al. compared the performance of accelerated solvent extraction and QuEChERS for the suitability to extract eleven drugs spiked in lettuce from a local supermarket [7]. Both optimized methods provided satisfactory extraction recovery and precision to allow for quantification of the pharmaceuticals in vegetable tissues. Compared to the accelerated solvent extraction method, the QuEChERS method provided better performance for the determination of drugs in vegetables in terms of ease, speed, and solvent consumption [7]. In contrast, the comparison of solid-liquid extraction with QuEChERS for the analysis of 28 wastewater-borne contaminants and their potential metabolites in lettuce reported better performance parameters for the former method [9]. In a recent study with a broader range of analytes [14], covering as many as 74 micro-contaminants, some of which were not previously investigated, extraction with QuEChERS yielded to satisfactory results. Up to 84 % of the compounds were recovered within a 70 to 120 % range.

The detection of the analytes in the aforementioned studies was accomplished with compound-specific acquisition on triple-quadrupole mass spectrometers (QqQ-MS) operated in a targeted mode. Recently, the development of very fast data acquisition modes in high resolution-mass spectrometry (HR-MS) on quadrupole-time of flight (Q-TOF) instruments has enabled novel approaches offering rapid and reliable results for a large number of compounds in a target acquisition mode. The so-called high resolution multiple reaction monitoring (MRM^{HR}) is a robust targeted quantitation mode through two stages of mass selection, to provide high data richness and excellent specificity and sensitivity. First-, the quadrupole mass filter selects a given precursor ion, fragments it by collision-induced dissociation, and then the user choses among the products ions one that provides the best combination of sensitivity and selectivity for quantification. As for all of the analytes, full $HR\text{-}MS^2$ spectra are recorded, their identities can be confirmed by checking them for the presence of additional fragment ions of diagnostic value. Conversely, some new Q-TOF hybrid systems have gained wide acceptance thanks to the Sequential Window Acquisition of All Theoretical Fragment-Ion Spectra (SWATH mode) providing high quality MS/MS

data that can be used for quantitation with fast acquisition speed and excellent mass accuracy [19, 20] SWATH is a data-independent acquisition technique, separating into fixed or variable size m/z windows stepped across the entire m/z range of interest. In this way, fragment ions formed in a given window cause more easily associated to their precursor ion, resulting in high specific MS and MS/MS spectra [21]. Although the main applications are proteomics and metabolomics [22-24], SWATH acquisition generates comprehensive and high-quality MS/MS spectra comparable to "MRM-like" fragments that can be used to confirm unequivocally the detection of specific compounds after comparing the SWATH data with pre-assembled MS/MS spectral libraries [23, 25].

Under this scenario, the main objective of the present study was to compare the performance of two high resolution mass spectrometry modes namely MRM^{HR} and SWATH using LC-QToF-MS for the determination of 48 wastewater-borne pollutants (including analgesics, antibiotics anti-inflammatories, antifungal agents anti-hypertensives, antimycotics, β-blockers, industrial pollutants, lipid regulators, psychiatric drugs and stimulants and sweeteners) in lettuce. Moreover, we also developed and validated an analytical method based on the QuEChERS extraction of lettuce leaves for the final determination of wastewater-borne pollutants of widespread use and commonly present in reclaimed water. The performance of 16 different modified QuEChERS procedures (with formic acid and PSA clean up step) to extract the selected analytes from this matrix were compared. After validation, the optimized analytical method was applied to the analysis of the selected compounds in lettuce plants grown in soil pots under controlled conditions and irrigated with treated wastewater for the whole crop cycle. Both HRM^{HR} and SWATH acquisition were achieved using a hybrid QTOF mass spectrometer

2. Materials and methods

2.1 Chemicals and reagents

Analytical reference standards (Acesulfame, acetaminophen, acridone, benzotriazole, 5-methyl-2H-benzotriazole, bezafibrate, bisphenol A, caffeine, carbamazepine, carbamazepine-10,11-epoxide, chloramphenicol, ciprofloxacin, citalopram, clarithromycin, climbazole, clofibric acid, diclofenac, 4'-hydroxydiclofenac, diltiazem, fenofibrate, fipronil, fipronil desulfinyl, fipronil sulfone, fluconazole, furosemide, gemfibrozil,

hydrochlorothiazide, ibuprofen, indomethacin, irbesartan, lamotrigine, lamotrigine N2-oxide, 5-desamino 5-oxo-2,5-dihydro lamotrigine, metoprolol, metronidazole, N2-methyl-lamotrigine, N-acetyl-sulfamethoxazole, 4-nitro-sulfamethoxazole, oxcarbazepine, propranolol, sucralose, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfanilic acid, valsartan, valsartan acid, and verapamil) were of high purity and were acquired from Sigma Aldrich (St. Louis, MO, U.S).

Isotope-labelled compounds (acetaminophen-d4, acesulfame-d4, benzotriazole-d4, bezafibrate-d4, bisphenol A-d8, caffeine-13C3, carbamazepine-d10, ciprofloxacin-d8, citalopram-d6, climbazole-d4, diclofenac-13C6, fenofibrate-d6, fluconazole-13C3, furosemide-d5, gemfibrozil-d6, hydrochlorothiazide-d2, ibuprofen-d3, indomethacin-d4, irbesartan-d6, lamotrigine-13C3, metoprolol-d7, metronidazole-d4, naproxen-d3, sucralose-d6, sulfamethazine-d4, sulfamethoxazole-d4, valsartan acid-d4, valsartan-d3) were purchased from Cerilliant (Sigma Aldrich, St. Lous, MO, U.S), Alsachim (Illkirch-Graffenstaden, France), Santa Cruz Biotechnology (Dallas, TX, US.), or Toronto Research Chemicals (Toronto, ON, Canada).

CAS numbers, molecular formulas, molecular weight, and other relevant properties of all target compounds are reported in Table A.1, (Appendix).

For standards and samples preparation, LC-MS grade acetonitrile (≥99.9%), methanol (≥99.9%), ethyl acetate (≥99.9%), dimethyl sulfoxide (≥99.9%), and HPLC water were purchased from Merck (Darmstadt, Germany). Formic acid (≥96%, ACS reagent), ammonium acetate ($NH_4CH_3CO_2$), and ammonium formate (NH_4HCO_2) were supplied by Sigma-Aldrich while ammonium fluoride was bought from Fisher Chemical (Fisher Scientific SL, Madrid, Spain). For high purity mobile phase solutions, acetonitrile and water (Optima™ LCMS Grade) were purchased from Fisher Chemical (Fisher Scientific SL, Madrid, Spain).

QuEChERS extraction salts and dispersive solid phase extraction (dSPE) were obtained from BEKOlut GmbH & Co. KG (Hauptstuhl, Germany). The Original non-buffered kit was composed by 4 g $MgSO_4$ and 1 g NaCl, while the buffered European EN 15662 kit was constituted by 4 g $MgSO_4$; 1 g NaCl; 1 g sodium citrate; 0.5 g disodium citrate sesquihydrate.

The dSPE clean-up mixture was made of 150 mg PSA (primary secondary amine), 150 mg of C18-bonded silica, and 900 mg $MgSO_4$.

2.2 Preparation of standard solutions

Stock solutions (1000 μg mL^{-1}) of individual pharmaceuticals standards were prepared in either acetonitrile, methanol, dimethylsulfoxide, or HPLC water depending on the solubility of each compound and stored in the dark at -20 °C. Working mixtures of pharmaceuticals and the isotopically labeled compounds (2 μg mL^{-1}), used for spiking the lettuce blank samples during the method development, in the validation studies, and for calibration purposes were prepared by diluting an appropriate volumes of the stock solutions in methanol.

2.3 LC-MS/MS analysis

Samples were analyzed on a SCIEX X500R QTOF system (Sciex, Redwood City, CA, U.S.) equipped with Turbo V™ Electrospray Ionization (ESI) source. Depending of the analytes, they were detected in negative or positive polarity mode. The total chromatographic run time for each injection was 12 min for positive or negative acquisition and the separation of the analytes was achieved on a Hibar HR Purospher STAR RP- C18 column (100 mm × 2.1 mm i.d., 2-μm particle size, Merck, Darmstadt, Germany), maintained at 40 °C. The fast elution was carried out using as mobile phases consisting of aqueous mobile phase (A), either 5 mM ammonium acetate + 0.1% formic acid (positive ion mode) or 2 mM ammonium fluoride (negative mode), and (B) acetonitrile. The flow rate was 0.5 mL/min, the injection volume was 10 μL, and the auto-sampler temperature was 8 °C. The elution gradient is reported in Table A.5.

Any possible drift in the mass accuracy of the SCIEX Q-TOF-MS was automatically corrected during batch acquisition by infusing a reserpine solution ($C_{33}H_{40}N_2O_9$, m/z 609.28066) for positive mode, and a cluster of trifluoroacetic acid ($[(CF_3COONa)^5$+ CF3COO]$^-$, m/z 792.85963) for negative mode. The instrument provided a typical resolving power (FWHM) of 31,000 to 44,000 at m/z 132.9049 and 829.5395, respectively with a mass error of 0.2 ppm. Calibration was performed before or after a control vial in the batch sequence making use of the Calibrant Delivery System (CDS).

All HR-MS data were acquired using either MRM^{HR} or SWATH modes. Quantitation was performed in the MRM^{HR} fragment scanning mode which provides the noise in the chromatogram to the minimum due to the selection of specific ions at specific collision energies (CE), decluttering potentials (DP), and fragmentation voltages (V). The SWATH acquisition in turn, lacked the selectivity of MRM^{HR} but the MS data set could be used for retrospective analysis.

Both modes consisted of a single TOF-MS experiment over a range from m/z 100 to 950 with an accumulation time (AT) of 120 ms; DP and CE were set to 80 V and 10 V and -80 V and -10 V, for positive and negative, respectively). The source conditions were as follows: source temperature and nitrogen gas flows (Atomizing gas, GS1 and Auxiliary gas, GS2) were set to 550° C, 55, and 55 psi, respectively. Ion Spray Voltage was set to 5500 V (-4500 V for negative); Collision gas (CAD) was set to 7, while Curtain gas was set to 30 psi. The MRM^{HR} experiments were acquired in fragment scanning mode. The Guided MRM^{HR} tool from SCIEX was used for the optimization of transitions. The selected ionization mode, the optimized CEs and Vs for each compound have been reported in electronic supplementary material (Table S2). The SWATH acquisition consisted of 10 MS/MS experiments with variable Q1 window widths (m/z 100 to 950, 40 ms AT) using a CE of 35V with ±15V spread. The variable Q1 windows were generated using the SCIEX SWATH variable window calculator (Ver. 1.1). The MS survey scan obtained for lettuce extract spiked with all the compounds was run in the window calculator to generate the variable window widths, for positive and negative acquisition. The outcomes are reported in Figure A.1.

Qualitative analysis was performed using SCIEX OS™ Software version 1.6 (Sciex, Redwood City, CA, USA). Two ions were used for each compound, the most abundant product ion for the quantification and the precursor ion for the confirmation (Table S2, ESM). Only the accurate mass of molecular ion obtained from the TOF-MS experiment was used for the isotopically labeled compounds. For SWATH acquisition, high confidence identification was based on unique fragment ions and their ion ratios as well as HR-MS/MS library searching using high resolution spectral libraries supplied by SCIEX.

2.4 Sample preparation

Lettuce (*Lactuca sativa* L. "Maravilla de Verano-Canasta") was selected as the matrix of this study for its fast growth, its high cultivation and consumption worldwide, its ability to grow easily in greenhouse conditions, and its extensive root system that can facilitate the uptake of organic contaminants from soil. Furthermore, it is usually consumed without being cooked and its vegetative part consists of green leaves, making the sample preparation easier [1]. To produce sufficient of contaminant-free matrix for method optimization and validation, several lettuce seedlings at the four leaf stage were grown for 60 days using organic potting soil purchased from a local garden store (Barcelona, Spain) [26]. At the harvest, lettuce plants were carefully hand washed with tap water and then rinsed with purified water. The heads then, were separated from the roots and blotted dry with a paper tissue and stored at -20°C for at least 48 h. The lettuce leaves were freeze-dried, using a LyoAlfa 6 system (Telstar Technologies, Terrassa, Spain) and ground to a fine powder with a knife mill with a stainless steel grinding chamber (Grindomix GM 200, Retsch GmbH, Haan, Germany) and stored at -20°C until extraction.

2.5 Extraction and clean up

The recovery studies were performed using a modified QuEChERS approach which was optimized by evaluating different extraction and clean-up conditions. The Original non-buffered (OR) and the European EN 15662 method (EN) QuEChERS extraction salts kits were compared. To assess the influence of acidification (formic acid) in the extraction efficiency, different concentrations were added to the extraction solvent (0.5 and 1%). To avoid the risk of base-catalyzed degradation following the use of PSA, acidification was also evaluated after the cleaning phase by adding 0.05% formic acid [27]. The efficiency of removal of undesirable co-eluents by the use of dSPE PSA-C18 clean-up was also tested. Finally, the alternative use of ammonium acetate and ammonium formate solutions instead of water during hydration step was also evaluated to improve the recoveries of challenging compounds. Protocols of the different extraction procedures are reported in Table 1 and described in details in the electronic supplementary material (Table A.6 and Table A.7) and discussed in Results section.

Table 1. Different procedures of modified QuEChERS performed, including all variants tested during the study.

	Type of salt		Hydration			Formic acid		Cleanup	
	ORIGINAL	EN 15662	HPLC water	Ammonium acetate	Ammonium formiate	0.5 %	1 %	PSA-C18	PSA-C18 0.05 % Formic acid
Protocol 1	■		■						
Protocol 2		■	■						
Protocol 3			■				■		
Protocol 4		■	■				■		
Protocol 5			■					■	
Protocol 6		■	■					■	
Protocol 7	■		■				■	■	
Protocol 8		■	■				■	■	
Protocol 9	■		■						■
Protocol 10	■		■			■			
Protocol 11	■		■			■		■	
Protocol 12	■		■			■			■
Protocol 13	■			■		■			
Protocol 14	■			■		■		■	
Protocol 15	■				■	■			
Protocol 16	■				■	■		■	

The streamlined procedure provided below which was adopted for extraction and clean-up in the final method. Briefly, 1 g freeze-dried blank lettuce was placed in 50-mL disposable polypropylene centrifuge tube and 9 mL HPLC water, (90% hydration). The tubes were vortexed for 2 min at 2500 rpm using a BenchMixer XLQ QuEChERS Vortexer (Benchmark Scientific, Sayreville NJ, US). After a 1-hour hydration phase, the sample was spiked with 50 µL of standard solution containing all target compounds (2 µg mL^{-1} in methanol) to achieve a final concentration in the lettuce of 100 ng g^{-1} dry weight (d.w.), corresponding to 10 ng g^{-1} of fresh weight (f.w.) after hydration step. The tube was vortexed again and the sample was allowed to stand for 30 min. Then 10 mL acetonitrile and 50 µL of concentrated formic acid were added followed by a vortex. The OR extraction salts were added directly into the tube and the mixture was instantly shaken in order to prevent the formation of

crystalline agglomerates owing to $MgSO_4$ hydration. The sample was vortexed again and centrifuged (4000 rpm, 10 min, 4 ° C). The resulting supernatant (organic phase) was transferred into a glass tube and left overnight at -20°C, enabling the precipitation of fatty acids and waxes, co-extracted from the lettuce leaves. The following day, the d-SPE clean-up took place. While avoiding to re-suspend the material deposited on the bottom, 6 ml of the organic phase were transferred into the PSA tube (150 mg PSA, 150 mg C18, 900 mg $MgSO_4$) and the mixture was shaken for 1 min manually, vortexed for 2 min, and centrifuged at 4000 rpm for 5 min, 4°C. Then, 1 mL of the supernatant was evaporated until dryness under a gentle stream of nitrogen at room temperature and then reconstituted with 1 mL of water/10 % methanol solution and injected for LC-MS/MS analysis.

2.6 Method performance

The analytical method was validated for specificity, accuracy, intraday precision, linearity, limits of detection (MDLs) and quantification (MQLs), and matrix effect (ME) using spiked lettuce samples.

To ensure the quality of the results, the specificity of the method was evaluated by analyzing untreated lettuce samples. The absence of signal above signal-to-noise ratio (S/N) of 3 at the retention time of the analytes of interest eliminates a false positive by contamination in the extraction process. The accuracy was determined with spiking the matrix at concentrations of 2, 5, 10, 50, and 200 ng g^{-1} f.w. Relative recoveries (R%) were calculated by comparing the peak areas obtained in samples spiked before the extraction (n=3) and after the extraction (n=3) at five concentration levels, according to equation 1:

$$R\% = 100 \times (\text{Area spiked pre extraction}) / (\text{Area spiked post extraction}) \quad (1)$$

The precision of the essay expressed by repeatability (intra-day) was calculated as relative standard deviation (RSD %) obtained from the relative recoveries of the recovery study for each concentration level. Values were considered Acceptable when recoveries were ranged between 70-120% and RSDs $\leq$ 20%. Both MRM^{HR} and SWATH acquisition were compared in term of accuracy and precision. Mean values of accuracy and precision for MRM^{HR} and SWATH are reported in Table 2.

In order to assess the ME extracts of untreated lettuce samples (n=3 replicates) were spiked with the mix of the target compounds at the same concentration levels as used in the recovery study (2, 5, 10, 50, and 200 ng g^{-1} f.w.), before LC-MS/MS analysis. The peak areas produced by these samples were compared with those obtained the solvent (water/10 % methanol,) spiked at equivalent concentrations. ME (%), were expressed according to the following equation 2:

$$ME\ (\%) = 100 \times [(Area\ in\ spiked\ extract / Area\ in\ spiked\ solvent) - 1] \quad (2)$$

Hereinafter, ME values of ±40 % were considered acceptable, whereas ME values outside this range indicated significant matrix effect.

To compensate for ME and to evaluate the linearity of the method, a matrix-matched calibration curve approach was employed. An 11-point calibration curve was prepared by spiking blank lettuce extracts with proper amounts of standard solution are a ranged 0.05 to 300 ng mL^{-1}, corresponding to 0.5 and 3000 ng g^{-1} d.w. in lettuce leaves. Each concentration was injected three times on the same day and the calibration curve was constructed by plotting the ratio of the analyte signal to its surrogate standard signal against the analyte concentration. Calibration curve was constructed by linear weighted least-squares regression (1/x as weighting factor). For the majority of the compounds, at least 8 calibration points were considered. Linearity was evaluated by calculating the coefficient of determination (r^2) for each analyte. The acceptance criterion was that the coefficient of correlation ≥0.99. Surrogate standards used in each case are shown in Table A.2.

Method detection limit (MDL) was defined as the lowest concentration of an analyte that could be distinguished of the matrix signal with a S/N greater than 3. Method quantification limit (MQL) was defined as the lowest concentration of a given compound giving a response that could be quantified, with a S/N greater than 10 and a RSD ≤ 20 %. MDL and MQL were estimated from the matrix-matched calibration curves based on the following equations according to [28]:

$$MDL = 3 \times S_b / slope \quad (3); \qquad MQL = 10 \times S_b / slope \quad (4).$$

where S_b is the standard deviation of the intercept.

2.7 Clean-up efficiency

To determine the amount of co-extracts removed in the matrix through the 2-step cleanup, a gravimetric measurement was conducted according to [29]. Nine 50-mL Falcon tubes were prepared weighting 1 g of blank lettuce matrix in each tube. Three samples were extracted using only the OR QuEChERS kit according to optimized protocol. Then, 5 mL of the organic supernatant layer (5 g equivalent) was transferred into three previously weighted test tubes. Three more samples were extracted in the same way but they were left overnight at -20°C, to enable the precipitation of fatty acids or waxes. The following day, taking care not to pick up the material deposited on the bottom, 5 mL of the organic phase (5 g equivalent) were transferred into 3 other pre-weighed glass test tubes. 5 mL (5 g equivalent) of each extract after freezing out and clean-up (150 mg PSA, 150mg C18, 900 mg $MgSO_4$) were transferred to pre-weighed test tubes. All nine test tubes were then evaporated until total dryness under a gentle stream of nitrogen at room temperature until constant weight. The difference in weight was recorded to estimate the amount of matrix co-extracts in the initial and final extracts. The results are reported in Figure A.2.

3. Results and discussion

Aiming to optimise the extraction of target compounds contaminants from lettuce leaves, the Original QuEChERS protocol developed by Anastassiades et al. for pesticide residues in food commodities [27] now involves the extraction of wastewater-borne compounds with acetonitrile containing 0.5 % of formic acid and simultaneous liquid–liquid partitioning formed by adding anhydrous $MgSO_4$ and NaCl. A two-step clean-up including dSPE was performed. The acquisition of HRMHR and SWATH were compared and, therefore, the optimal conditions were selected. Finally, some parameters were studied that influence the performance and efficiency of the extraction including the matrix effect.

3.1 Method performance: Optimization of LC-MS/MS conditions

In the present study, 3 replicates, each at 5 spiking levels (2, 5, 10, 50, and 200 ng g^{-1} f.w.), in lettuce leaves were prepared using the selected QuEChERS protocol and analyzed by LC-

QToF-MS. The best chromatographic separation of molecules of interest was performed with a Merck Hibar HR Purospher STAR RP-C18 column. Separation of all studied target analytes was successfully performed, and this column was chosen for further analysis. To achieve the optimal separation and high sensitivity for MS detection gradient elution based on mobile phase consisting of acetonitrile and water (5 mM ammonium acetate + 0.1% formic acid) for the positive electrospray ionization and acetonitrile and water (2 mM ammonium fluoride) for the negative ionization, at a flow rate of 0.5 mL min^{-1}. For development and comparison experiments, only the MRM^{HR} approach was used by acquiring data in fragment scanning mode. The Guided MRM^{HR} tool from SCIEX was used for the optimization of high resolution transitions. High resolution MRM parameters including ionization mode, CEs and Vs were carefully studied for each compound to provide the best possible sensitivity. Both electrospray ionization ESI+ and ESI- modes were tested. According to SANTE European Commission guideline for pesticides (SANTE/11813) [30], two ions with mass accuracy equal or mass difference lower than 5 ppm are necessary for confirming a positive finding for the identification in HR-QToF-MS analysis. In the present study, each compound was confirmed by comparing the signal of two high resolution ions, the most abundant product ion with the best signal intensity for the quantitation while the precursor ion for the confirmation. Results of the optimized mass spectrometric conditions in MRM^{HR} for each compound are shown in Table A.2. In contrast, for SWATH acquisition a fixed value of DP of 80 V and -80 V, for positive and negative, respectively with a CE of 35 V with a collision energy spread of ±15V was employed since they are essential for comparison with the high resolution spectral libraries supplied by SCIEX. In order to estimate its sensitivity, the validation of targeted compounds was also performed acquiring data with SWATH acquisition and afterwards both modalities were compared. For an accurate quantitation, the MRM^{HR} fragment scanning mode provided high selectivity and sensitivity of product ion transitions, decreasing the noise in the chromatogram to the minimum due to the use of specific DPs and CEs. On the other hand, the information acquired through the SWATH mode can always be useful at a later time for querying the data to identify new unidentified metabolites through a retrospective analysis. In fact, SWATH mode combines in the same run high quality HR-MS and HR-MS/MS data that can be used for quantitation or for a retrospective analysis. To enhance the selectivity [25], the SWATH Variable Window

Calculator was used to optimize the Q1 isolation window pattern for the matrix of interest, in this case lettuce, to achieve the right balance of compound coverage and specificity. Ten sequential Q1 variable windows were generated by injecting a lettuce matrix sample fortified with all target compounds in full-scan. The obtained MS survey scan contains the list of the m/z values of all precursors and the intensities from all the peaks detected in the spectrum within the same retention time window of the chromatographic gradient. Variable windows were generated by computing the number of precursor ions and taking into account their intensities as a weighting factor. The generated windows based on the precursor ion distribution within the retention time of the LC gradient for both positive and negative SWATH acquisition were reported in supplementary material (Figure A.1).

Instrumental and method performances were assessed by considering accuracy, intra-day precision linearity, and limits of detection and quantitation. Data can be found in Table 2. Both MRM^{HR} and SWATH acquisition were compared in term of recoveries and precision. In term of accuracy, most of the compounds exhibited similar relative recoveries between 80 and 120 % for both methods. However, not all compounds were adequately recovered. In fact, few compounds showed poor recoveries such as sulfanilamide, ciprofloxacin, and sulfamethazine presenting values of 26.8, 27.8, and 28.4 % in MRM^{HR} and 25, 33.9, and 35 % in SWATH, respectively. Moreover, indomethacin was recovered only at 50 and 200 ng g^{-1} in SWATH acquisition (Table A.3). The intra-day precision expressed by repeatability was calculated as relative standard deviation (RSD%) obtained from the relative recoveries studied for each concentration level. Most of compounds are very precise with a deviation less than 10%, below the recommended 20 % for both acquisition methods. Only one compound was >20 % (acetaminophen, 22.3 %) for MRM^{HR}, while in SWATH just three compounds presented an inaccuracy higher than 20 % (Gemfibrozil 20.3 %, irbesartan 20.8 % and ibuprofen 24.4 %). Individual values of relative recoveries and precision for each concentration for MRM^{HR} and SWATH are reported in Table A.3. Recovery values are in line with values reported elsewhere in similar methods [14, 31]. Interestingly, although the recoveries are quite similar between the two acquisition modes, some differences are observable in terms of linearity and correlation coefficient. The linearity of the method was assessed using the matrix-matched calibration approach with a calibration curve constructed between 0.05 and 300 µg L^{-1} (equivalent to 0.5-3000 ng g^{-1} d.w. of lettuce leaves) taking into

account at least eight calibration points. Calibration curves were constructed using linear weighted least-squares regression (1/x as weighting factor) by plotting the ratio of the analyte peak area to that of its corresponding IS. In MRM^{HR}, the chromatographic response was linear up to 2000 ng g^{-1} for most compounds covering several order of magnitude with correlation coefficients (r^2) above 0.99 for most compounds (Table 2). Only ibuprofen and fipronil desulfinil presented r^2 <0.98, with values of 0.9628 and 0.9669, respectively. Despite the reliable results of MRM^{HR}, SWATH acquisition provided an overall shorter linearity response for all compounds with values up to 500 ng g^{-1} for most compounds. This may be due to the detector which in this case must manage a multitude of ions at the same time and for this reason it is saturated. Furthermore, due to a reduction in orders of magnitude, r^2 was also affected. More in details, r^2 ranged from 0.9699 to 0.9995 for all compounds except for fipronil sulfone, N2-Methyl-Lamotrigine, and fipronil desulfinyl presenting r^2 of 0.9258, 0.9472, and 0.9542, respectively. In addition, linearity and r^2 for ibuprofen could not be calculated, given that this compound generally requires a quite large accumulation time that was possible only working in MRM^{HR}.

Regarding sensitivity, the Method Detection Limits (MDLs) and Method Quantification Limits (MQLs) were estimated from the matrix-matched calibration curves using linear regression analysis. As the Table 2 shows, both methods reported overlapping limits between them for the majority of compounds. For MRM^{HR}, MDLs and MQLs ranged from 0.01 to 0.12 ng g^{-1} and 0.04 to 0.38 ng g^{-1}, respectively. Unfortunately, for SWATH, despite most of the compounds presented acceptable MDLs and MQLs ranging between 0.01 to 0.16 and 0.04 to 0.50 ng g^{-1}, respectively, eight compounds (sucralose, benzotrialzole, sulfanilic acid, hydrochlorothiazide, acetaminophen, citalopram, fenofibrate, and metronidazole) have provided higher values (MDLs and MQLs ranging between 0.17 to 1.74 and 0.52 to 5.28 ng g^{-1}, respectively).

3.2 Evaluation of matrix effect

Lettuce leaves are a very complex matrix, and its extraction by aqueous organic solvent mixtures often leads to the presence of co-extracted matrix components in the final extracts [1, 32-34]. Although it is necessary to extract a wide range of analytes as efficiently as

possible, these co-extractive components of the matrix are not desired and may alter the ionization efficiency of the analytes in the ionization source influencing the signal intensity due to competition for available charged ions [35]. In fact, co-extractives can dramatically influence analysis performance causing suppression or improvement of the analyte response by decreasing or increasing the instrumental response factors of the target analytes, compared to those observed in the solvent [33, 34]. In the quantitative analysis, ME can negatively influence an accurate quantification especially according to the type of sample and the properties of the analyte [36]. The mean ME of the five spiking levels for all compounds comparing both acquisition modes (MRM^{HR} and SWATH) are reported in Figure 1 whereas they are well detailed in Table A.4.

To reduce the number of co-extracts and decrease the effects of the matrix, the use of a cleaning phase during sample preparation is often indispensable. [1, 37-39]. As a result, besides recoveries, the impact of matrix effect (ME) was also assessed to evaluate the effect on the response of the analytes. The extent of matrix effects was measured by comparing the response in blank lettuce extracts and in solvent spiked at the same concentration levels used for recovery study (2, 5, 10, 50, 200 ng g^{-1}), before LC-MS/MS analysis. The extent of the effects due to the components of the matrix classified according to the average ME% for each compound is shown in Figure 1 and is well detailed for each level in Table A.4. Both MRM^{HR} and SWATH acquisition have given similar effects matrix between them regardless of the instrumental conditions used. In fact, as reported in Figure 1, the patterns observed are quite overlapping with respect to the matrix, indicating low effect (from -40 to 40 %) for the 50 % of the investigated compounds. ME values outside the acceptable range of |40| % denote strong signal suppression or enhancement with a consequent impact on method performance. For instance, the most susceptible are compounds with acidic groups (i.e. valsartan, fenofibrate, gemfibrozil, and sulfanilic acid) resulted the compounds more affected by the ion suppression close to -90 % for both MRM^{HR} and SWATH. In contrast, sucralose and clofibric acid showed substantial enhancement for MRM^{HR} (98.6 and 135 %, respectively) while oxcarbazepine and acetaminophen for SWATH (109.4 and 212.6 %, respectively). Curiously, acetaminophen shows an opposite performance depending on acquisition method used (-78.3 % for MRM^{HR} and 212.6 % for SWATH). Since co-eluted substances of the matrix may reduce the ion intensity of the target compounds, the use of a matrix-matched

calibration curve combined with the internal standard approach should solve this problem, improving the accuracy of the quantification and reducing the signal suppression/enhancement of the analyte.

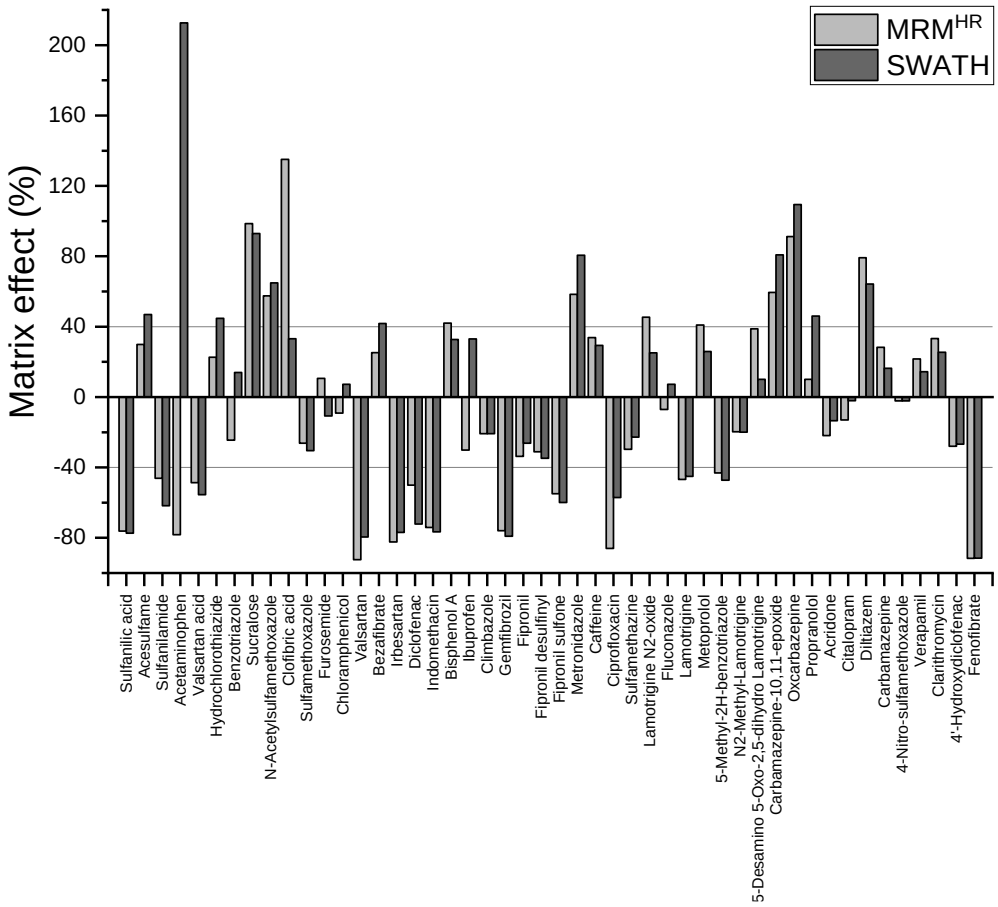

Figure 1. Comparison of Matrix effect (%) for target compounds in different acquisition modes (MRM^{HR} and SWATH) at five different spiking levels. Bars refer to the mean values of the five concentrations. Compounds are ordered by retention time and ionization mode. From sulfanilic acid to fipronil sulfone for negative ionization, and from metronidazole to fenofibrate for positive ionization.

3.3 Optimization of the sample extraction procedure: comparison of different methods

Freeze drying process prolong the stability of compounds of interest in commodities with a high amount of water without causing a sensible reduction in their amount [40]. For this reason, we decide to use freeze-dried lettuce with a low residual water for the development of the present method. However, the original QuEChERS method was designed for fresh

samples of 10 g with more than 80 % water [27]. To keep the salting out as similar as possible to the original method with the use of 10 g of fresh plant tissue and due to the normal water content of a lettuce greater than 90%, we have chosen to use 1 g of freeze-dried lettuce leaves and fix the hydration volume to 9 ml of water (ammonium acetate or ammonium formate).

The effect of salt and buffer addition, on the partitioning of the compounds was preliminary evaluated comparing two commercially available kits (OR and EN). In this first screening, a dispersive SPE clean-up using PSA/C18 and/or the addition of 1 % of formic acid were also evaluated (Table A.6). The OR kit resulted more effective allowing the recovery of the 62 % of the compounds (R% >60 %) compared to EN (only 55 %) (Figure A.3). The addition of formic acid prior to the cleanup step seems to slightly reduce the overall recoveries. For example, benzotriazole, caffeine, carbamazepine-10,11-epoxide, and furosemide resulted significantly influenced by the use of the acid (Table A.6). Since the pka of these compounds is > 7.5, in an acidic pH, it would already be positive charged and the extraction in the organic solvent is less efficient [41]. Also the clean-up step tends to reduce the recovery of some analytes. In particular, the PSA, being a weak anion exchanger, could affects the recovery of more polar organic compounds due to hydrophilic interactions. [31]. The combined use of 1 % HCOOH and PSA/C18 leads to a slightly increase in recoveries especially in the case of OR protocol (67 %). The EN buffer seems to mitigate this effect (55 %). In this first evaluation OR 1 % HCOOH PSA/C18 appeared the best choice.

In follow-up experiments, the efficiency of the selected QuEChERS method (OR) was assessed by comparing the addition of a proper amount of HCOOH in combination of PSA and alternative hydration solvents. In this second phase, three new compounds, of which the analytical standards were not available at the beginning, were added (5-desamino 5-oxo-2,5-dihydro Lamotrigine, lamotrigine N2-oxide, and N2-methyl-lamotrigine). Suitable modifications of the OR method were compared in Figure 2. The Original QuEChERS and Original PSA/C18 were compared against the addition of 50 μL formic acid (0.5 %). Due to the prolonged contact with PSA [27], the pH of the extracts could increase compromising the stability of some pharmaceuticals with acidic groups (e.g. fenofibrate, ibuprofen, indomethacin) and reduce the overall recoveries. Hence, the possible degradation of such basic compounds was also tested by an immediate acidification of the extracts after the

PSA/C18 step by adding 10 μL of a 5 % formic acid solution in acetonitrile per mL (corresponding to a final concentration of 0.05 % HCOOH). This step was tested with or without the initial acidification (Figure 2). The addition of acid after the cleanup step seems to adversely affect some compounds such as ciprofloxacin, indomethacin, and valsartan (Table S7, ESM). The addition of ammonium formate or ammonium acetate were also tested to enhance the extraction efficiency. Both solutions were prepared by adding 200 mg in 9 mL of pure water with a final pH of 6.6 and 6.7, respectively. These ammonium salts, when added during the hydration of the sample, are supposed to compete with the target compounds improving the absorption of interfering substances on the sorbent. As a result, the pure water replacement should induce phase separation and extraction due to a lower adsorption of the target analytes to the matrix [42]. However, the addition of ammonium salts during the hydration showed that no relevant improvement was observed when we used ammonium acetate. About 9 % of compounds were not recovered in presence of ammonium acetate, whether or not PSA is employed (Figure 2). On the other hand, when we used ammonium formate a significant decrease of the average recoveries was indeed observed. In fact, about 20 % of the compounds have not been recovered at all when OR is used without PSA. This percentage is reduced to 11 % in the presence of PSA, however insufficient to justify its use. Also in this case, the most susceptible compounds are ciprofloxacin and some drugs with acidic groups (fenofibrate, ibuprofen, indomethacin) (Table A.7). Finally, the addition of 50 μL formic acid (0.5 %) to the Original QuEChERS following by a PSA/C18 clean-up step seems to provide significant benefits to justify the change of our initial addition of 1% formic acid. The use of 0.5% HCOOH led to improvement in recoveries enabling the highest recoveries of all compounds. In this way, this combination was more effective given that all the compounds were effectively recovered reporting values above 20 %. In particular, 76 % of the compounds presented values higher than 60 %. (Figure 2 and Table A.7). Only 4 compounds presented values below 40 % (ciprofloxacin 21.8 %, sulfamethazine 20.5 %, sulfamethoxazole 23.9 %, and sulphanilamide 38.7 %). Based on the results of the experiments described in Figure 2, Original HCOOH 0.5 % PSA/C18 was selected for the further validation study.

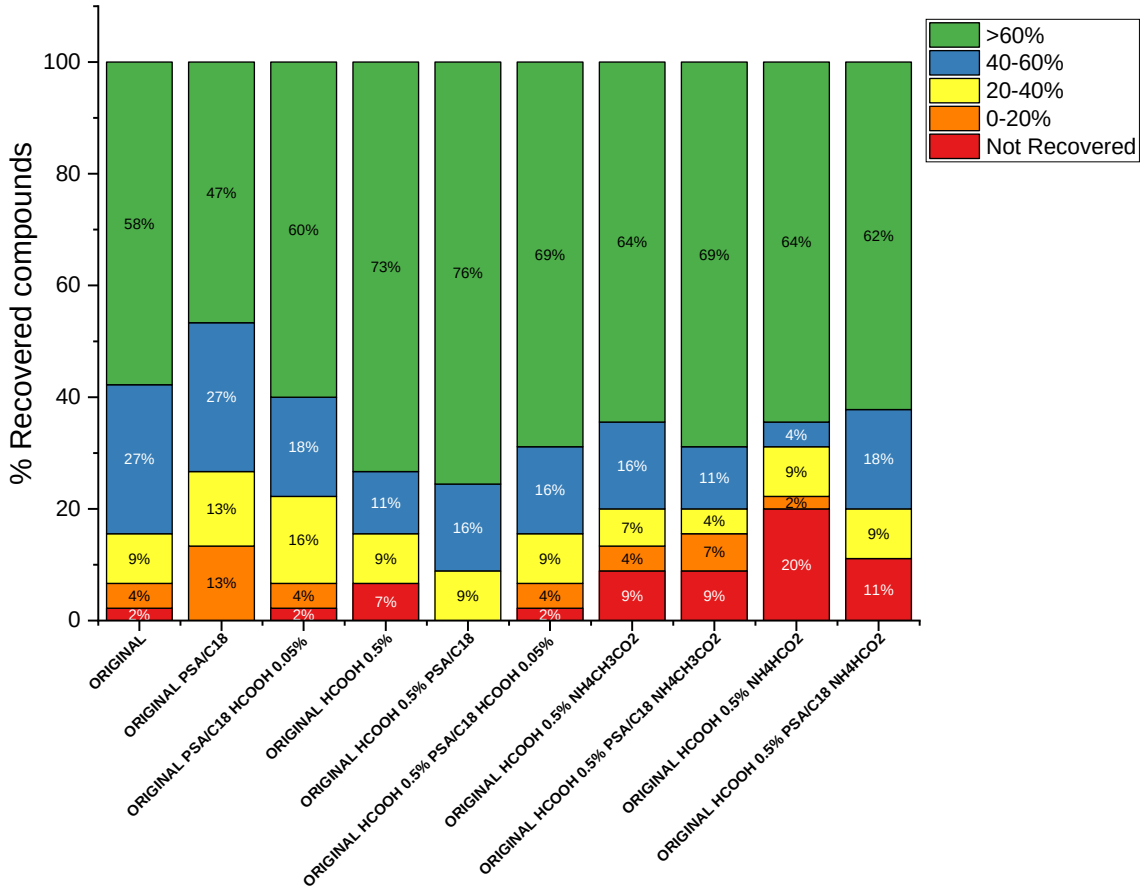

Figure 2. Comparison of recoveries (%) of target analytes in lettuce spiked at 10 ng g^{-1} using the Original QuEChERS extraction salts kit and different modifications of the Standard method including the hydration of the sample, and/or the clean-up step.

3.4 Effect of 2-step clean-up on co-extractives

The amounts of undesirable co-extractives from lettuce leaves samples were determined by weighting after evaporation of the extracts to dryness in pre-weighted test tubes, according to optimized protocol. In general, the use of acetonitrile in the partitioning step minimized only the fat co-extractives [19, 27]. The dSPE clean-up with PSA was not initially developed to remove chlorophyll and sterols from vegetable extracts [43]. In fact, the PSA can only retain fatty acids and other polar compounds in the matrix due to the presence of the primary and secondary amine moieties. Furthermore, the reverse phase absorbent C18 is able to effectively remove starch and sugar from samples by trapping them [44]. A dispersive SPE

formed of a combination of PSA and GCB (Graphitized Carbon Black) is generally used to remove chlorophyll from samples with a high content of pigments [27]. To prevent the compounds of interest from being absorbed by the GCB, we opted for an additional simple and inexpensive cleaning procedure which consists in leaving the extracts overnight at -20 ° C (freezing-out) before the following clean-up step with dispersive PSA. This approach that does not require extra solvents such as hexane [1], which is generally used to promote the precipitation of fats at to the low temperature [19], and can be used to remove co-extractives in bulk from aqueous solvents or other relatively polar solvents [45]. Figure A.2.A shows the amount of co-extractives in the acetonitrile extracts using only OR protocol, the effect of temperature, and the reduction of co-extractives in presence of the 2-step clean-up. The gravimetric results demonstrated the reduction of the amount of co-extractives in acetonitrile extracts by almost 60 % by weight using the freezing-out step. In addition, after the dispersive-SPE clean-up, another large part of co-extractives was removed from extracts. The freezing procedure together with the dispersive clean-up led to a rather substantial reduction of co-extractives (-83.5 %) corresponding to a residual amount of 5 mg in the final extract. Although it has not been evaluated, the addition of formic acid seems to favour the precipitation of chlorophyll during freezing-out, as shown in Figure A.2.B. Another important advantage of the freezing-out is that part of the residual water that $MgSO_4$ was unable to remove during the salting out, is deposited on the bottom of the tube, frozen together with the co-extractives.

3.5 Application to Real Samples

In order to evaluate the presence of pharmaceuticals in lettuce leaves, the validated method was applied to lettuce plants growth in controlled condition (greenhouse experiment). During the study, 12 lettuce seedlings (*Lactuca sativa* L.) cv. Maravilla de Verano-Canasta at the approximately four-leaf stage were transplanted in 12 plastic pots (22 cm diameter) filled with 3 kg of pristine soil collected from the Parc Agrari of El Prat de Llobregat (Barcelona, Spain). For the first five days after transplant, all plants were irrigated with tap water. Afterwards, eight plants were irrigated with 100 mL of treated wastewater effluent provided by the wastewater treatment plant EDAR of El Prat de Llobregat (Barcelona, Spain) each two days for the entire growing period. The remaining four pots were used as controls and

were irrigated with 100 mL of tap water each two days. After 60 days, all plants were harvested, washed with deionized water to remove any soil residues, and gently blot dried with paper towel. Then, the samples were extracted according to the optimized procedure.

Due to less trustworthy results of SWATH, quantification of the target analytes in plant samples was based on MRM^{HR} and was performed by the internal standard method. Each analyte was quantified by using its corresponding deuterated standard. An isotopically labelled compounds with similar retention time or from the same group was used only for the quantification or those compounds in which isotopically analogues compounds were not available (Table A.2). Only 14 out of 48 studied compounds were detected and were reported in Figure 3. Most of detected compounds presented concentrations up to 10 ng g^{-1}, such as metoprolol (2.9 ng g^{-1}), bisphenol A (3.4 ng g^{-1}), climbazole (3.3 ng g^{-1}), fluconazole (3.4 ng g^{-1}), diltiazem (4.6 ng g^{-1}), carbamazepine (6.0 ng g^{-1}), and valsartan acid (9.1 ng g^{-1}). Five compounds were detected in the leaves at concentrations close to 50 ng g^{-1}, like irbesartan (13.4 ng g^{-1}), caffeine (15.0 ng g^{-1}), carbamazepine epoxide (18.1 ng g^{-1}), sulfamethazine (33.2 ng g^{-1}), and hydrochlorothiazide (45.6 ng g^{-1}). Only two analytes were found at very high concentration: gemfibrozil (185 ng g^{-1}) and citalopram (196 ng g^{-1}), although the former has a rather important variability. These results are comparable with those previously reported by other authors working with the same matrix [5, 7, 9, 46]. Irrigation with reclaimed water or contaminated water containing trace levels of pharmaceuticals could lead to uptake and the consequent accumulation of pharmaceuticals in green parts of lettuce crops, posing potential risks to human health.

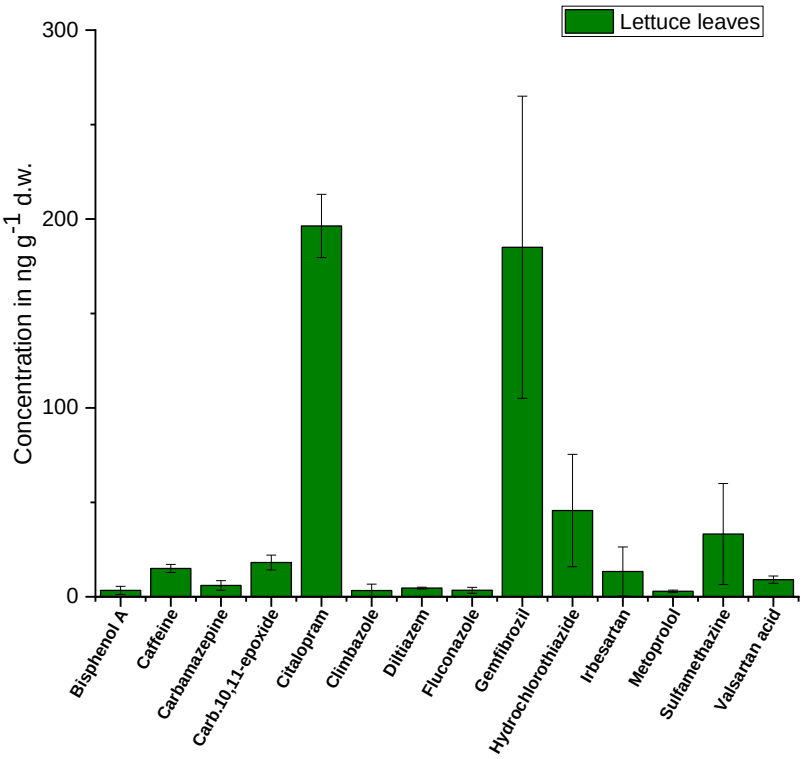

Figure 3. Presence of target compounds in lettuce samples irrigated with treated wastewater effluent.

4. Conclusions

Based on the results of this study, we concluded that the simple changes made to the classic QuEChERS method provided good possibilities to achieve our goals and simultaneously improve the overall recoveries for a large number of compounds without sacrificing the performance of a multi-residual method. Method performances were also studied by comparing two different acquisition techniques provided by the same instrument. Although the two techniques provide quite similar results in term of accuracy and limits of detection, higher and more consistent results for a greater number of analytes were achieved using MRM^{HR} acquisition. However, we reserve to discover in a future study the full potential of SWATH mode not only to quantify small molecules but also to explore its capabilities in suspect screening and non-target analysis in a so complex matrix like lettuce, taking full advantage of high resolution and high resolution spectral libraries.

Table 2. Method performance parameters for target analytes in MRM^{HR} and SWATH acquisition including Retention time (RT), Linearity range, Coefficient of determination (r^2), Accuracy (%), Precision (RSD, %), and Method detection limits (MDL) and Method quantitation limits (MQL).

		MRM^{HR} Linearity		SWATH Linearity		MRM^{HR} Intraday performance		SWATH Intraday performance		MRM^{HR}		SWATH	
Analyte	**RT (min.)**	**Range (ng g^{-1})**	**r^2**	**Range (ng g^{-1})**	**r^2**	**Accuracy[a] (%)**	**Precision[b] (RSD, %)**	**Accuracy[a] (%)**	**Precision[b] (RSD, %)**	**MDL[c] (ng g^{-1})**	**MQL[d] (ng g^{-1})**	**MDL[c] (ng g^{-1})**	**MQL[d] (ng g^{-1})**
4'-Hydroxydiclofenac	7.54	2.5 - 2000	0.9921	5 - 3000	0.9935	83.3	17.5	78.9	13.7	0.09	0.26	0.05	0.15
4-Nitro-sulfamethoxazole	6.86	2.5 - 2000	0.991	1 - 500	0.9958	92.6	10.8	90.9	5.4	0.06	0.18	0.06	0.18
5-Desamino 5-Oxo-2,5-dihydro Lamotrigine	4.64	5 - 2000	0.984	2.5 - 1000	0.997	80.8	19.0	80.1	6.9	0.08	0.25	0.04	0.12
5-Methyl-2H-benzotriazole	4.41	2.5 - 2000	0.9927	2.5 - 2000	0.9972	100.	14.5	89.6	13.0	0.05	0.16	0.04	0.12
Acesulfame	1.18	2.5 - 3000	0.9873	2.5 - 1000	0.9955	65.6	9.0	72.4	7.6	0.12	0.38	0.08	0.25
Acetaminophen	2.01	5 - 3000	0.9954	5 - 2000	0.9985	84.6	22.3	85.8	7.0	0.04	0.13	0.67	2.04
Acridone	5.82	0.5 - 1000	0.9886	5 - 500	0.987	96.5	11.4	93.6	2.9	0.02	0.05	0.08	0.25
Benzotriazole	3.23	0.5 - 3000	0.9953	0.5 - 1000	0.993	91.1	10.7	95.6	16.4	0.06	0.18	0.18	0.54
Bezafibrate	5.38	1 - 1000	0.9866	1 - 500	0.9946	90.8	4.9	91.6	6.5	0.03	0.09	0.05	0.15
Bisphenol A	7.39	1 - 3000	0.9904	2.5 - 2000	0.9914	90.9	6.1	89.8	3.4	0.08	0.26	0.05	0.16
Caffeine	2.82	1 - 3000	0.9956	10 - 2000	0.9954	87.6	9.9	90.4	5.4	0.02	0.07	0.10	0.29
Carbamazepine	6.42	5 - 1000	0.9988	0.5 - 500	0.9958	99.3	4.1	96.8	1.9	0.06	0.18	0.02	0.06
Carbamazepine-10,11-epoxide	5.32	0.5 - 2000	0.9947	1 - 500	0.9995	97.2	10.2	97.8	4.9	0.04	0.11	0.02	0.06
Chloramphenicol	5.18	0.5 - 1000	0.9938	0.5 - 500	0.9913	90.4	9.4	94.8	3.2	0.02	0.06	0.02	0.06
Ciprofloxacin	3.54	2.5 - 2000	0.9914	2.5 - 2000	0.9797	27.8	9.2	33.9	12.4	0.01	0.04	0.07	0.22
Citalopram	6.22	1 - 1000	0.9935	1 - 1000	0.9818	89.1	7.2	90.2	4.4	0.07	0.21	0.68	2.05
Clarithromycin	7.22	2.5 - 2000	0.9963	2.5 - 1000	0.982	88.8	4.1	90.6	3.2	0.01	0.04	0.04	0.12
Climbazole	8.39	1 - 1000	0.9917	1 - 500	0.9746	86.7	10.4	85.8	8.8	0.05	0.14	0.08	0.25
Clofibric acid	4.07	2.5 - 3000	0.9911	1 - 1000	0.9951	90.3	9.5	89.0	4.8	0.02	0.05	0.06	0.18
Diclofenac	6.95	5 - 2000	0.9917	2.5 - 1000	0.9894	87.0	14.6	90.6	14.5	0.05	0.17	0.08	0.23
Diltiazem	6.37	2.5 - 2000	0.9945	2.5 - 1000	0.9917	93.9	4.7	94.7	4.1	0.04	0.11	0.04	0.11
Fenofibrate	9.89	1 - 1000	0.9956	2.5 - 1000	0.9946	83.7	18.4	90.4	16.0	0.06	0.20	1.63	4.93
Fipronil	8.92	0.5 - 100	0.9988	0.5 - 100	0.9706	95.7	6.4	95.1	5.8	0.03	0.08	0.04	0.14
Fipronil desulfinyl	9.05	2.5 - 100	0.9669	0.5 - 50	0.9542	94.9	4.1	94.6	4.3	0.02	0.05	0.02	0.07
Fipronil sulfone	9.22	1 - 100	0.9888	0.5 - 50	0.9258	93.3	6.1	93.1	7.7	0.01	0.04	0.02	0.05
Fluconazole	4.02	2.5 - 3000	0.9957	2.5 - 2000	0.9947	90.3	5.6	92.3	3.6	0.02	0.07	0.03	0.10
Furosemide	4.42	1 - 3000	0.999	5 - 1000	0.9974	64.1	7.9	68.9	5.3	0.11	0.32	0.16	0.50

Gemfibrozil	8.78	1 - 3000	0.9937	2.5 - 500	0.9699	87.6	11.2	96.0	20.3	0.08	0.26	0.08	0.23
Hydrochlorothiazide	2.77	2.5 - 1000	0.9867	2.5 - 500	0.9864	87.5	9.1	99.5	11.8	0.06	0.18	0.44	1.33
Ibuprofen	7.68	10 - 2000	0.9628			106.0	16.6	101.7	24.3	0.07	0.22		
Indomethacin	7.24	2.5 - 2000	0.9888	5 - 2000	0.9858	92.6	8.3	105.6*	12.7*	0.10	0.31	0.12	0.37
Irbesartan	6.49	1 - 1000	0.9902	1 - 500	0.9865	79.3	16.5	81.0	20.8	0.03	0.08	0.03	0.09
Lamotrigine	4.02	2.5 - 1000	0.9926	2.5 - 500	0.9912	65.5	4.5	66.7	7.8	0.05	0.14	0.01	0.04
Lamotrigine N2-oxide	3.91	5 - 1000	0.9835	2.5 - 1000	0.999	81.3	9.3	79.9	5.9	0.03	0.09	0.05	0.14
Metoprolol	4.17	2.5 - 2000	0.9989	0.5 1000	0.9986	86.2	5.3	87.6	6.9	0.09	0.27	0.10	0.29
Metronidazole	2.16	5 - 2000	0.9953	1 - 1000	0.9913	86.6	14.0	92.3	10.2	0.10	0.32	1.74	5.28
N2-Methyl-Lamotrigine	4.62	2.5 - 1000	0.9945	5 - 500	0.9472	87.1	5.1	87.3	7.9	0.03	0.09	0.09	0.28
N-Acetyl-sulfamethoxazole	3.72	1 - 1000	0.9945	2.5 - 1000	0.9945	86.5	4.8	89.1	7.2	0.04	0.12	0.11	0.35
Oxcarbazepine	5.6	5 - 2000	0.9905	5 - 1000	0.9968	55.5	6.6	62.2	4.5	0.04	0.11	0.07	0.22
Propranolol	5.64	2.5 - 2000	0.9951	1 - 1000	0.9984	92.9	7.1	103.1	8.9	0.08	0.24	0.04	0.13
Sucralose	3.35	5 - 3000	0.9809	10 - 3000	0.9718	78.9	7.7	77.2	5.4	0.10	0.30	0.17	0.52
Sulfamethazine	3.62	2.5 - 2000	0.9918	2.5 - 3000	0.9993	28.4	7.5	35.0	7.2	0.07	0.22	0.05	0.16
Sulfamethoxazole	4.16	1 - 2000	0.9928	1 - 1000	0.9957	40.5	8.3	46.3	7.7	0.04	0.11	0.07	0.20
Sulfanilamide	1.28	5 - 3000	0.9951	2.5 - 2000	0.9987	26.8	9.4	25.0	9.9	0.04	0.13	0.04	0.11
Sulfanilic acid	0.47	5 - 3000	0.9946	10 - 2000	0.9789	60.9	10.3	52.7	9.9	0.08	0.25	0.25	0.76
Valsartan	5.37	2.5 - 2000	0.9952	5 - 2000	0.9962	81.1	17.2	73.5	18.7	0.05	0.15	0.09	0.27
Valsartan acid	2.54	5 - 2000	0.9928	2.5 - 1000	0.9991	40.7	14.5	44.0	14.3	0.03	0.08	0.06	0.17
Verapamil	7.1	1 - 1000	0.9933	0.5 - 500	0.9888	92.8	7.9	94.6	4.9	0.03	0.09	0.02	0.06

[a] Accuracy was expressed as mean of relative recoveries calculated from the five studied levels.

[b] Precision was calculated as average relative standard deviation (RSD %) obtained from the relative recoveries at each concentration level.

[c] MDLs were estimated from the matrix-matched calibration curves using linear regression analysis.

[d] MQLs were estimated from the matrix-matched calibration curves using linear regression analysis.

* Indomethacin was recovered only at 50 and 200 ng g^{-1} in SWATH acquisition.

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Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Nicola Montemurro*: Conceptualization, Methodology, Writing - Original Draft, Validation, Data analysis

Anastasia Orfanoti: Experimental work, Writing - Original Draft

Rayana Manasfi: Experimental work, Writing - Original Draft, Software

Nikolaos S. Thomaidis: Revision & Editing

Sandra Pérez: Conceptualization, Supervision, Project administration, Writing - Revision & Editing, Funding acquisition

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