IX SIMPOSIO de Investigación EN CIENCIAS EXPERIMENTALES

PESTICIDE RESIDUE RESEARCH GROUP

VALIDATION OF A QUICK AND EASY EXTRACTION METHOD FOR THE DETERMINATION OF EMERGING CONTAMINANTS AND PESTICIDE RESIDUES IN AGRICULTURAL SAMPLES

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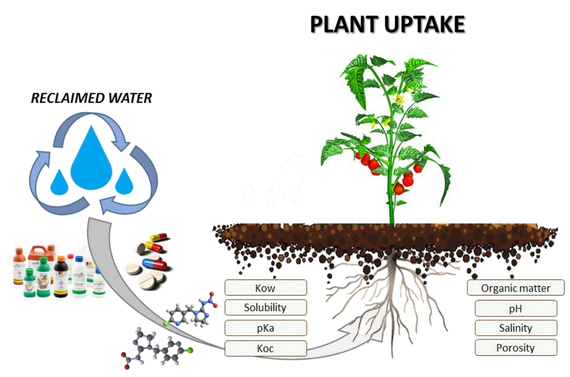
INTRODUCTION

The use of reclaimed wastewater for agricultural irrigation is increasing worldwide. This practice is becoming an alternative to combat water scarcity. However, it is known that this water can contain multiclass of organic microcontaminants because the removal procedures are deficient. Some studies have reported that using reclaimed water for irrigation can lead to the accumulation of some organic microcontaminants in soil and crops grown using reclaimed water. Thus, the aim of this study was to develop and validate a quick and easy extraction method based on the QuEChERS method for the determination of 31 organic contaminants in agricultural samples. Finally, the analytical methodology developed was applied to measure the uptake and translocation of the selected organic compounds in soil, leaves and fruit, in a tomato crop grown in a greenhouse under controlled conditions.



EXPERIMENTAL

Selected compound

Family	Compound	Log Kow	pKa	koc (mL/g)	Solubility in water (mg/L)
Antibiotic	Ofloxacin	-0,39	5,97	44,143	28300
Analgesic	4-AAA *	-0,13	12,84	-	40226
Stimulant	Caffeine	-0,07	14,00	741-7762	21700
Diuretic	Hydroclorotiazide	0,07	7,90	12	722
β–βλοχκερ	Atenolol	0,16	9,60	-	13300
Analgesic	4-FAA *	0,17	12,72	-	101289
Antibiotic	Ciprofloxacin	0,28	6,09	61000	30000
Analgesic	Paracetamol	0,46	9,38	20844	14000
Analgesic	4-AA *	0,47	4,07	-	727617
Analgesic	4-MAA *	0,61	-	-	28897
Analgesic	Codeina	1,19	8,21	700	0,577
Antiepileptic	Carbamazepine-10,11Epoxi	1,58	-	-	1340
Analgesic	Diclofenac	1,9	4,15	245	2,37
Diuretic	Furosemide	2,03	3,90	110	73,1
Antiepileptic	Carbamazepine	2,45	13,90	510	17,7
Anti-inflammatory	Naproxeno	3,18	4,15	330	15,9
Fibrate	Gemfibrozil	4,77	4,50	430	11
Fibrate	Fenofibric Acid	5,19	3,10	-	5,1
Fungicide	Carbendazim	1,51	4,20	122-2805	8
	Thiabendazol	2,39	4,73	2500-4680	30
	Azoxystrobin	2,50	0,93	210-580	6,7
	Fluxapyroxad	3,08	12,60	496-1424	3,44
	Myclobutanil	3,17	2,30	950	115
	Penconazole	3,72	1,51	786-4120	73
Herbicide	Diuron	2,87	13,60	55-962	37,4
Insecticide	Pymetrozine	-0,19	4,06	246-7875	270
	Thiamethoxam	-0,13	0,41	32-237	4100
	Imidacloprid	0,57	11,12	156-800	610
	Acetamiprid	0,80	0,70	132-267	4250
	Thiacloprid	1,26	0,50	408-1584	185
	Diazinon	3,30	2,60	191-1842	60

** metabolites (metamizole): 4-metilaminoantipirina (4-MAA), 4-aminoantipirina (4-AA), 4-formilaminoantipirina (4-FAA), and 4-acetill-amino-antipirina (4-AAA)*

Pilot studies

- Under controlled conditions
- Greenhouse´s surface: 540 m^2
- Contaminated reclaimed water: 1 µg/L
- The crops were watered daily for 15 min.
- Total amount of water: 2,200 L.
- Irrigation water samples were taken once a week from the exit of the drips.
- Vegetable samples were collected once the crop was finished (3 months).
- Soil samples were taken of the upper 10 cm layer.

LC-QqQ-MS/MS analysis

Chromatography

Sciex Exion HPLC system

- Column: C8 Agilent 100 mm x 2.1 mm (1.8 µm)
- Mobile phase:
  - a) H_2O (0.1 % CH_2O_2)
  - b) AcN
- Flow: 0,3 mL/min
- Total run time: 18 min

MS parameters

Sciex 6500+ TripleQuand system

- ESI (+/-) mode
- Acquisition mode: dMRM
- Curtain gas: 20
- GS1: 50 psi
- GS2: 40 psi
- Gas temperature: 500 °C
- Capillary voltage: 5000 V
- Injection volume 10 µL

Extraction methods

10 g of sample

+ 5 mL MiliQ-H_2O

10 mL AcN (0.5% v/v, formic acid) + 10 µL IS (1)

Shake automatically 6 min

4 g $MgSO_4$ anh. + 1 g $NaCl_4$ + 1 g $Na_3Citrate{\cdot}2H_2O$ + 0,5 g Na2HCitrate·1,5H2O

Shake automatically 6 min

Centrifugue 5 min 3500 r.p.m.

Take 5 mL aliquot

125 mg PSA +

750 mg $MgSO_4$ anh.

+ 150 mg ChloroFit + 125 mg PSA

+ 125 mg C18

Shake 30 second/Vortex

Centrifugue 5 min 3500 r.p.m.

50 µL formic acid 5% in AcN + IS2

LC-QqQ-MS/MS Analysis

(1) Malathion d10, Carbendazim d3, Caffeine ^{13}C; (2) Dimethoate d6)

RESULTS

The optimized procedure were evaluated according to the European Union quality control guidance document in term of selectivity, sensitivity, linearity, repeatability and trueness. **Figure 1** shows some of results such as limit of quantification (LOQ), matrix effect (ME) or recovery in term of percent.

1. Most target compound shown LOQs ≤ 0.1 ng/g in all matrices studied (soil, leaf and fruit).
2. No matrix effect (≤20%) was observed in tomato. Only 29% and 3% of selected compounds presented a high ME in leaf and soil matrices, respectively.
3. Recovery studies were evaluated from the spiked sample at different levels per triplicate. More than 60%, of target analytes shown values over 70%.
4. Intra and inter-day precision (repeatability/reproducibility) values were between 0% and 9%.

Figure 1. *Results of LOQ, ME and recovery in term of percent*

Optimized methods were applied in a case-control study carried out on the experimental farm of UAL-ANECOOP located in Almería (Spain).

The total concentration of pesticides detected in tomato samples was 3 µg/kg whereas the pharmaceuticals total load was 6.4 µg/kg. In the soil samples, the concentration levels found after crop harvesting were up to 11.4 µg/kg for pesticides and 2.3 µg/kg for pharmaceuticals (see **Figure 2**)

Figure 2. *Total concentration of pesticides and pharmaceuticals detected in the different part of plant (µg/Kg)*

CONCLUSIONS

- **The method developed provided excellent sensitivity (≤ 0.1 ηg/g), repeatability (1-22%), good recoveries (70-120%), and low matrix effects (≤ 20%) for most selected compounds. It can be used as a useful tool for the simultaneous determination of a multiclass of organic contaminants with different physical-chemical properties in agricultural samples.**
- **The concentrations of organic microcontaminants in the edible part (fruit) of the plants were below 10 µg/kg in all cases, even when the irrigation condition can be considered as a worst-case of contamination by pesticides.**
- **The results obtained under greenhouse climatic conditions highlighting the importance to carry out analysis of the agricultural soil samples to avoid high pesticide accumulation that could be migrated during further crop seasons.**

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