MethodsX 8 (2021) 101290

Contents lists available at ScienceDirect

MethodsX

journal homepage: www.elsevier.com/locate/mex

Method Article

Validation of a quick and easy extraction method for the determination of emerging contaminants and pesticide residues in agricultural soils

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A B S T R A C T

Treated wastewater is currently used in the agricultural sector to solve the lack of availability of freshwater in many regions. However, reclaimed water can contain multiclass of organic contaminants. Therefore, the soil can become a reservoir of agricultural (e.g. pesticides) and urban (e.g. pharmaceuticals) contaminants. Consequently, the evaluation of this contamination process is necessary for assessing its potential human and environmental negative effects.

Due to the low concentration levels, different chemical properties and the complexity of the matrix, an efficient sample preparation step for achieving adequate sensitivity and robust analysis in the soil is needed.

The aim of this study was to develop a quick and easy extraction method based on a QuEChERS procedure for the determination of 27 organic contaminants in agricultural soil samples. The procedure was based on a salting-out extraction with acidified acetonitrile, followed by a dispersive solid-phase extraction (d-SPE). A liquid chromatography-tandem mass-spectrometry (LC-MS/MS) system was applied for the determination and quantification of the selected target analytes. The main benefits of this analytical approach are:

- Reduction/elimination of majority of the interferences improving the sensitivity of the method.
- Robust simultaneous determination of a multiclass of organic contaminants with very different physicochemical properties.

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https://doi.org/10.1016/j.mex.2021.101290

ARTICLE INFO
Method name: A version of the QuEChERS citrate-buffered method
Keywords: Pesticides, Pharmaceuticals, QuEChERS, LC-MS/MS, Reclaimed water, Agricultural soil
Article history: Available online 27 February 2021

Specification table

Subject area	Chemistry
More specific subject area	Analytical Chemistry
Method name	A version of the QuEChERS citrate-buffered method
Name and reference of original method	The citrate QuEChERS buffered method resulted in the European Standard EN 15662 (Foods of Plant Origin Determination of Pesticide Residues Using GC-MS and/or LC-MS/MS Following Acetonitrile Extraction/Partitioning and Clean-up by Dispersive SPE (QuEChERS method)) published in 2008 (www.cen.eu)
Resource availability	

Methods details

Chemicals and reagents

Analytes included in this study were selected based on previous experience [1]. They comprise a group of 27 organic contaminants belonging to different compound categories: 13 pesticides (herbicides, fungicides and insecticides) and 15 emerging contaminants (analgesics, anti-inflammatories, lipid regulators, β-blockers, antiepileptic and diuretics). All analytes were acquired from Sigma-Aldrich (Steinheim, Germany) at analytical grade (> 98%), except codeine that was obtained by dissolving a tablet (30 mg/pill). ^{13}C-caffeine, carbendazim-d3, malathion-d_{10} and dimethoate-d6 were selected as internal standards to check the extraction and analytical efficiency. Individual stock solutions were prepared at 1000 mg/L in AcN, except codeine that was prepared in a mixture water-methanol (50:50, v/v at pH = 10). These solutions were stored at −40°C in amber screw-capped glass vials. Working solutions were daily prepared by dilution with water of these individual solutions. HPLC-grade acetonitrile was purchased from Merck (Darmstadt, Germany) and LC-MS optima grade water from Fisher Scientific (Fair Lawn, NJ, USA). Formic acid (purity 98%) was obtained from Fluka (Buchs, Germany). Magnesium sulphate anhydrous ($MgSO_4$), sodium hydrogen citrate sesquihydrate ($Na_2HCitrate{\cdot}1{,}5H_2O$), sodium citrate tribasic dihydrate ($Na_3Citrate{\cdot}2H_2O$) and sodium chloride (NaCl) were purchased from Sigma-Aldrich (Steinheim, Germany). Bondesil-C18 sorbents was obtained by Agilent Technologies (Santa Clara, CA, USA).

Sample collection and pretreatment

Development and validation of the analytical method was carried out using agricultural soil samples collected in April 2020 from an experimental farm of UAL-ANECOOP located in Almería (Spain). The soil contained 15% clay, 20% silt and 65% sand. It had 0.92% organic carbon, 100 ppm of total nitrogen, and pH and electrical conductivity values of 7.7 and 3240 µS/cm, respectively. Soil samples of the upper 10 cm layer were collected in polyethylene bags and transferred to the laboratory where they were sifted with a 2 mm diameter and dehydrated in an oven at 30°C for 24 h.

Modified QuEChERS method

Sample extraction was based on a previously published method by our research group [2], with some modifications. Soil sample (10 g) was hydrated with 5 mL Milli-Q water and extracted with 10 mL AcN (0.5% v/v, formic acid). After, 10 µL of a mix of internal extraction standards at 10 mg/L was added to check the extraction efficiency (caffeine-13C, carbendazim-d3, and dichlorvos-d6). The sample was shaken in an automatic axial extractor (AGITAXs, CirtaLab.S.L., Spain) for 6 min at 25°C. Next, extraction sals (4 g of anhydrous $MgSO_4$, 1 g of Na3Citrate$\cdot 2H_2O$, 1 g of NaCl and 0.5 g of

$Na_2Hcitrate{\cdot}1{,}5H_2O$) were added and the sample was shaken once more. The sample was centrifuged at 3500 rpm for 5 min. Then, 5 mL of supernatant was transferred to a 15 mL polyethylene tube with 750 mg of anhydrous $MgSO_4$ and 125 mg of C18. Next, the sample was shaken with vortex for 30 s and centrifuged at 3500 rpm for 5 min. After centrifugation, 4 mL of the cleaned extracts were transferred to screw-cap vials. Before injection, 100 µL of the extract was evaporated to dryness under a nitrogen stream and reconstituted with 90 µL of AcN:water solution (1:9, v/v) and 10 µL of dimethoate-d6.

LC-MS/MS analysis

A Sciex Exion HPLC coupled to a Sciex 6500+ TripleQuand-LC-MS/MS from Sciex was used for the determination and quantification of target compounds. Chromatographic separation was performed on a Zorbax Eclipse Plus C8 of 1.8 µm x 2.1 mm x 100 mm (Agilent). Mobile phases were 0.1% formic acid in water optim (solvent A) and AcN (solvent B) at a constant flow rate of 0.3 mL/min. The optimized gradient program was: 10% of B (initial conditions) for 0.5 min, after a linear gradient up to 100% of B in 11.5 min; kept at 100% of B for 4 min and finally, the mobile phase came back to the initial conditions (10% B). The total run time was 18 min and the injection volume was 5 µL. The HPLC was coupled to a QqQ-MS/MS with an ESI source (turbo spray iondrive), operating with positive and negative ionization modes. The ionization settings used in the positive mode were: ionspray voltage, 5000 V; curtain gas, 20 (arbitrary units); GS1, 50 psi; GS2, 40 psi; and temperature, 500 °C. The working parameters in the negative mode were the same values as for positive mode except to ionspray voltage, −4500 V. In both cases, nitrogen was used as the nebulizer gas and collision gas.

Individual standard solutions of each analyte at 200 µg/L were used by the optimization of the MS parameters. These solutions were infused directly into the MS system in full-scan mode. The most intense ion was chosen as the precursor ion. Next, in the product-ion mode were selected the optimal CEs for the two most intense transition; the most intense ion was selected as the quantifier ion (SRM1) and the second ion as the qualifier ion (SRM2). Table 1 shows the optimal mass spectrometric parameters for each target compound. The identification criteria followed were set out at the SANTE Document [3]. These criteria were: The quantification transition (SRM1) with s/n $\geq$ 10; the detection transition (SRM2) with s/n $\geq$ 3; retention time $\pm$ 0.1 min with reference to standard and comparing of fragment ion area with precursor ion area (ion ratio) with a value $\pm$ 30%.

Validation of the analytical methodology

To ensure that the optimized procedure was suitable for the application in routine, the analytical performance parameters such as selectivity, sensitivity, linearity, repeatability, reproducibility and trueness were evaluated according to the European Union quality control guidance document [3]. Information about the analytical performance data for each target compound is presented in Table 2. Analyses of matrix-blank samples were performed to test interferences using the proposed analytical approach. The results were compared to those obtained with an aqueous solution of the analytes at concentrations near the limit of quantification (LOQs). No significant interference has been detected in the retention time of the compounds, indicated good selectivity of the analytical method. The sensitivity of the method was calculated in terms of LOQs. All studied compound had LOQs equal to 0.05 ng/g, except to 4-AAA, and naproxen which showed values of 0.1 ng/g, beside 4-FAA, acetaminophen and furosemide which showed values of 0.5 ng/g. The linearity and matrix effect were evaluated using areas obtained of calibration curves prepared in matrix and solvent at seven concentration levels from 0.05 to 50 ng/g (range from LOQ to one hundred times more). Results were satisfactory because no significant matrix effect ($\leq$ 20%) were observed, except to atenolol, azoxystrobin, carbamazepine, diazinon, fluxapyrosad, gemfibrozil and penconazole which showed moderate matrix effects (20–33%). the correlation coefficients (r2) were higher than 0.991 in all the cases. Recovery studies were evaluated from the spiked sample at 10 and 50 ng/g per triplicate. Fig. 1 shows the average recoveries at 50 ng/g for all target analytes. Matrix matched calibration curves were prepared in order to quantification purposes. Extraction recoveries were calculated by comparing the response of each analyte in the matrix-matched calibration curve with the

Table 1
Instrumental parameters for target emerging contaminants using LC-MS/MS.

Compound	RT (min)	ESI	precursor ion (m/z)	DP	SRM 1	CE 1	SRM 2	CE 2
Pymetrozine	1.09	+	218.1	60.0	105.0	30.0	78.0	58.0
Atenolol	1.38	+	267.1	56.0	190.1	27.0	145.2	35.0
Acetaminophen	1.88	+	152.1	60.0	110.1	20.0	65.0	35.0
Codeine	2.18	+	300.2	50.0	165.1	55.0	225.0	36.0
Carbendazim	2.21	+	192.0	41.0	160.1	25.0	132.1	41.0
Thiabendazole	2.47	+	201.8	82.0	175.1	37.0	131.1	45.0
Caffeine	2.67	+	195.0	76.0	138.0	27.0	110.2	31.0
Hydrochlorotiazide	2.96	–	296.0	−70.0	205.0	−32.0	269.1	−26.0
4-AAA	3.04	+	246.2	56.0	228.0	19.0	83.0	43.0
4-FAA	3.05	+	232.2	45.0	214.2	19.0	204.0	18.0
Thiamethoxam	3.68	+	292.0	41.0	211.0	19.0	181.0	33.0
Imidacloprid	4.34	+	256.1	26.0	209.0	23.0	175.0	27.0
Acetamiprid	4.66	+	223.0	46.0	126.1	27.0	90.0	49.0
Epoxide-CBZ	4.95	+	253.0	46.0	180.0	41.0	236.0	17.0
Thiacloprid	5.20	+	253.0	41.0	126.0	25.0	90.0	55.0
Carbamazepine	5.81	+	237.1	46.0	194.2	29.0	192.1	29.0
Furosemide	5.83	–	329.1	−45.0	285.1	−20.0	205.1	−29.0
Diuron	6.73	+	233.0	50.0	72.1	42.0	133.0	58.0
Naproxen	6.98	+	231.1	51.0	185.2	21.0	170.0	37.0
Azoxystrobin	8.00	+	404.0	56.0	372.0	21.0	329.0	35.0
Myclobutanil	8.03	+	289.1	51.0	70.0	35.0	125.0	29.0
Fluxapyrosad	8.13	+	382.0	95.0	361.9	19.0	342.0	20.0
Fenofibric acid	8.18	+	319.1	71.0	233.1	25.0	139.1	39.0
Diclofenac	8.25	–	294.0	−30.0	250.0	−16.0	178.0	−42.0
Penconazole	8.45	+	284.1	41.0	70.1	42.0	159.0	21.0
Gemfibrozil	9.06	–	249.2	−30.0	121.1	−19.0	127.1	−14.0
Diazinon	9.41	+	305.1	41.0	169.0	29.0	153.1	29.0

4-Acetamianoantipyrine (4-AAA); 4-Formylaminoantipyrine (4-FAA); RT: retention time; DP: declustering potential; CE: collision energy; CXP: collision cell exit potential (10 V/ −10 V); EP: entrance potential (10 V/−10 V).

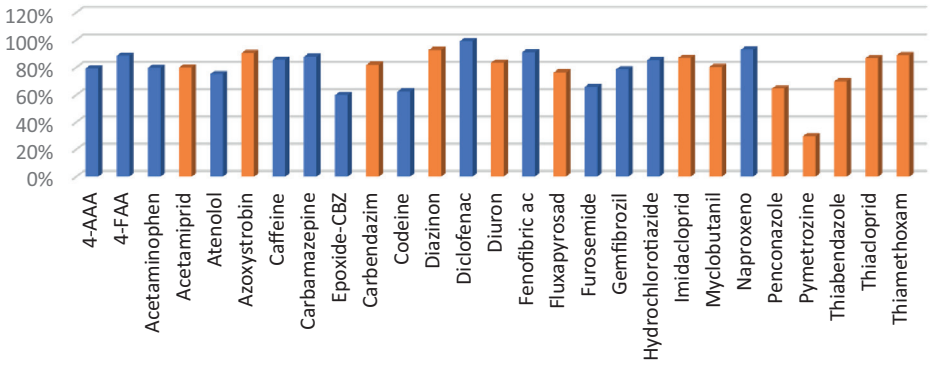

Fig. 1. Recoveries obtained at 50 ng/g for all target analytes. Pesticides (in orange); Emerging Contaminants (in blue) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

response detected in the spiked samples after the extraction. The average recovery values for both spike levels were higher than 70% for all analytes included in the study, except to carbamazepine epoxide (60–68%), codeine (47–63%), furosemide (63–66%) and pymetrozine (30%). Intra and inter-day precision (repeatability/reproducibility) was also calculated for each analyte from results obtained of the recovery study in terms of relative standard deviations (RSD,%). In both cases, the values were between 0 and 20%.

Table 2
Validation results obtained using the modifier QuEChERS extraction method developed.

Compound	LOQ (ng/g)	Linearity (r2)	ME (%)	Recovery (%)		Interday	Intraday
				10 ng/g	50 ng/g	RSD (%)	
4-AAA	0.10	0.9936	−7	73	79	4	19
4-FAA	0.50	0.9993	0	81	89	3	4
Acetaminophen	0.50	0.9990	−5	76	80	5	20
Acetamiprid	0.05	0.9983	−11	83	80	8	10
Atenolol	0.05	0.9981	−25	73	75	3	14
Azoxystrobin	0.05	0.9920	−33	71	90	5	6
Caffeine	0.05	0.9999	−6	72	86	1	16
Carbamazepine	0.05	0.9997	−26	79	88	6	3
Carbamazepine epoxide	0.05	0.9954	−13	68	60	3	10
Carbendazim	0.05	0.9978	−17	70	82	4	4
Codeine	0.05	0.9914	−7	47	63	5	1
Diazinon	0.05	1.0000	−34	84	93	4	6
Diclofenac	0.05	0.9973	−18	86	99	18	4
Diuron	0.05	0.9981	−11	79	83	2	6
Fenofibric ac	0.05	0.9992	−6	87	91	4	11
Fluxapyrosad	0.05	0.9991	−21	73	76	4	3
Furosemide	0.50	1.0000	2	63	66	15	7
Gemfibrozil	0.05	0.9994	−33	76	79	9	13
Hydrochlorotiazide	0.05	0.9997	1	85	85	5	18
Imidacloprid	0.05	0.9996	−16	86	87	2	15
Myclobutanil	0.05	0.9994	−17	81	80	20	10
Naproxen	0.10	0.9997	−17	82	93	3	9
Penconazole	0.05	0.9993	−30	73	65	7	19
Pymetrozine	0.05	0.9997	−6	30	30	1	12
Thiabendazole	0.05	0.9923	−15	62	70	7	19
Thiacloprid	0.05	0.9976	−20	82	87	3	6
Thiamethoxam	0.05	0.9993	−9	87	89	1	6

4-Acetamianoantipyrine: 4-AAA; 4-Formylaminoantipyrine:4-FAA; Limits Of Quantification (LOQs), Linearity expressed by the correlation coefficient, Matrix effect (ME) and Recovery (%) and Repeatability and Reproducibility expressed by the relative standard deviation.

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.

Acknowledgments

The authors are very grateful to the Spanish Ministry of Science, Innovation and Universities (MICINN) for the financial support given to the Project “POPOSITION" (CTM2017–89767-C3–3-R). M. García Valverde acknowledges the pre-doctoral Training Research Staff (FPI) fellowship program (PRE2018–087072). Dr. María del Mar Gómez Ramos acknowledges funding obtained for a research contract from the European Social Found 2014–2020 and the Regional Government of Andalucía. F.J Díaz Galiano acknowledges the pre-doctoral Training University Lecturer (FPU) fellowship program (grant FPU18/05113) awarded by the Spanish Ministry of Science, Innovation and Universities.

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