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Original Article

Comparison and evaluation of two methods for the pesticide residue analysis of organophosphates in yerba mate

Lucía Pareja[a], Silvina Niell[a], Zisis Vryzas[b,1], Joaquín González[c], María Verónica Cesio[a,c], Euphemia P. Mourkidou[b], Horacio Heinzen[a,c,*]

[a] Polo Agroalimentario y Agroindustrial, Departamento de Química del Litoral, Centro Universitario de Paysandú, Universidad de la República, Estación Experimental Mario Cassinoni, Ruta 3, Km 363, Paysandú, Uruguay
[b] Aristotle University of Thessaloniki, Pesticide Science Laboratory, P.O. Box 1678, 54124 Thessaloniki, Greece
[c] Farmacognosia y Productos Naturales, Departamento de Química Orgánica, Facultad de Química, UdelaR, General Flores 2124, 11800 Montevideo, Uruguay

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ABSTRACT

Microwave Assisted Extraction and a modified CEN-QuEChERS methodology were evaluated as extraction and clean up procedures for the simultaneous analysis of 42 organophosphate pesticides in yerba mate (*Ilex paraguaiensis*). The obtained extracts were analyzed by gas chromatography using a flame photometric detector. Linearity, recovery percentages, relative standard deviations, detection and quantification limits and matrix effects were determined according to DG-SANCO guidelines for both methods. At 0.2 and 0.5 mg/kg the evaluated methods showed percentages recoveries between 70 and 120% for most of the analytes. Using Microwave Assisted Extraction methodology, 33 pesticide residues could be properly analyzed whereas only 27 could be determined with the proposed modified QuEChERS. All relative standard deviation were below 18% except for omethoate and disulfoton sulfone when evaluated by the modified QuEChERS. The limits of detection in both methodologies were 0.2 mg/kg for most of the analyzed compounds. The average detection limit for QuEChERS was 0.04 mg/kg. For 19 of the analytes determined through Microwave Assisted Extraction the lowest validated level were 0.004 mg/kg. Signal suppression/enhancement was observed for most of the pesticides, thus matrix-matched calibration curves were used for quantification. The Microwave Assisted Extraction and QuEChERS procedures studied could detect the organophosphate pesticides above the MRL fixed for "mate" by the European Union. They have been successfully applied for the determination of organophosphate pesticide residues in commercial samples and the positives were confirmed through GC–(ITD)-MS.

Introduction

Ilex paraguariensis A. St.-Hil., Aquifoliaceae, is a native tree from the Rio de la Plata basin in South America. It has been cultivated since colonial times. Nowadays, 300,000 tons of processed leaves are consumed each year, which are used to prepare an infusion called Mate, the national beverage of Uruguay, Argentina, southern Brazil, and Paraguay. The art of mate drinking has been described by Pérez Parada et al. (2010), Jacques et al. (2007) and Vázquez and Moyna (1986). This traditional beverage is reputed to have a characteristic bitter taste and hepatoprotective, choleretic, hypocholesteremic, antioxidant, antirheumatic, diuretic and lipolitic properties (Filip et al., 2001).

As any other crop, yerba mate is attacked during farming by pests, especially mites, leaf-eating beetles and caterpillars forcing the use of organophosphate insecticides, that left pesticide residues. As yerba mate has been being sold steadily in Europe alone or in combination with other herbs as energy tea or as a weight reduction aid (Andrade et al., 2012; Heck et al., 2007) the European Union has established MRL of pesticide residues on the leaves (European Commission, 2005).

Yerba mate is a complex matrix for pesticide residues analysis due its chemical composition (natural pigments, lipids, vitamins and secondary metabolites: polyphenols, saponins, and xanthines like caffeine and theobromine) (Heck et al., 2007; Vázquez and

* Corresponding author at: Polo Agroalimentario y Agroindustrial, Departamento de Química del Litoral, Centro Universitario de Paysandú, Universidad de la República, Estación Experimental Mario Cassinoni, Ruta 3, Km 363, Paysandú, Uruguay.
E-mail: heinzen@fq.edu.uy (H. Heinzen).
[1] Present address: Democritus University of Thrace, Faculty of Agricultural Development, Laboratory of Agricultural Pharmacology & Ecotoxicology, 193, Pantazidou str. 68200, N. Orestiada, Greece.

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Moyna, 1986), and only few studies have been reported (Pérez Parada et al., 2010; Jacques et al., 2006). Particularly, caffeine and saponins are co-extracted with pesticides as they have similar physicochemical properties. Large amounts of caffeine and saponins contaminate the injector and the detector of the GC system, interfering with the determination of pesticide residues (Xu et al., 2011; Pérez Parada et al., 2010). The gas chromatographic separation of pesticides has been reviewed. Several analytical strategies and column types have been proposed for pesticide residue analysis in matrices such as tea, tobacco and herbs (Liu and Min, 2012; Khan et al., 2014).

The actual trend for pesticide residues determination at trace levels seeks for validated analytical methods with shorter analysis time and higher sample throughput (Chen et al., 2011). Considering mate a "tea-like" matrix, there are several methodologies reported for the analysis of pesticide residues in made tea, tea infusion and spent leaves. These methods include, for example, extraction with different solvents like ethyl acetate (EtOAc), cyclohexane or acetonitrile, combined with different clean up procedures; such as gel permeation, and solid phase clean up, either dispersive or using cartridges, followed by liquid or gas chromatography analysis, coupled to mass detectors (Huang et al., 2007, 2009; Kanrar et al., 2010). Lozano et al. (2012) and Cajka et al. (2012), described the application of a modified QuEChERS for the determination of pesticides in different types of teas. The QuEChERS approach is a very flexible one as it is a template to adapt the procedure according to analyte properties, matrix composition, equipment and analytical techniques available in the laboratory (Anastassiades et al., 2003). QuEChERS based methods have been used to asses food safety and environmental sustainability. Several reports on QuEChERS applications in herbs have been developed but there are no reports on QuEChERS for the analysis of pesticide residues in yerba mate leaves (Sadowska-Rociek et al., 2013; Attallah et al., 2012; Lozano et al., 2012; Chen et al., 2011, 2012a,b; Nguyen et al., 2010; Hayward et al., 2013).

Some other methodologies employing pressurized liquid extraction, dispersive liquid–liquid microextraction and dispersive solid phase extraction have been described in the literature for the analysis of pesticide residues in tea (Nguyen et al., 2010; Moinfar et al., 2009; Cho et al., 2008). Microwave assisted extraction (MAE) has been assayed as extraction and clean up procedure in food matrices (Vryzas et al., 2007; Papadakis et al., 2006; Vryzas et al., 2002), but there is no report for MAE in herbal teas. Its main advantages are low solvent consumption, short extraction time, and high level of automation with high extraction efficiency (Niell et al., 2011; Papadakis et al., 2006).

The present work compares MAE and QuEChERS performance for pesticide residues analysis of yerba mate leaves.

Materials and methods

Analytical standards and pesticide grade solvents were from Promochem (Wesel, Germany), Riedel-de Häën (Seelze, Germany) and Merck (Darmstadt, Germany). Anhydrous magnesium sulphate ($MgSO_4$), Graphitized Carbon Black (GCB) and ENVI-carb SPE, cartridge and PSA (primary–secondary amine) were from Sigma–Aldrich (Madrid, Spain). Sep-Pak silica cartridges were from Waters Corporation (Milford, MA, USA), PSA sodium citrate dibasic sesquihydrate and sodium citrate tribasic dihydrate were supplied from Supelco (Bellefonte, PA, USA).

Stock solutions of individual analytes at 1 mg/ml were prepared in EtOAc; three mixed standard stock solutions were prepared and serially diluted with EtOAc to produce a series of working standard solutions of 0.001–20 mg/l. The latter solutions were used for the construction of calibration curves and the preparation of the fortified samples. Stock solutions were stored in deep freeze (−23 °C), while the working standard solutions were stored refrigerated and renewed at weekly intervals. Matrix-matched calibration solutions (0.05–4 μg/ml) were prepared drying 0.2 ml yerba mate extract under a N_2 stream and fortified with 0.2 ml working standard solutions of pesticides at various concentrations. These matrix-matched solutions were used to prepare calibration curves, to evaluate the linear range, and to calculate recoveries.

Apparatus

The MSP 1000 laboratory microwave system (CEM, Matthews, NC) equipped with 12 vessel carousel with temperature and pressure sensors, operated in the closed mode was used for the microwave assisted extraction (MAE) of yerba mate leaves. PTFE-lined extraction vessels were used.

Pesticide residues analysis was performed in a Thermo Fisher Scientific, model Finnigan Trace GC (Rodano, Milan, Italy), gas chromatograph equipped with a flame photometric detector (FPD), an autosampler (model AS 3000), and a Programmed Temperature Vaporizer (PTV) (initial temperature was 60 °C (hold for 1.5 min) then increased to 220 °C at the rate of 5 °C/s for 35 min). The GC oven had two capillary columns in tandem (BP-1, 10 m, ID 0.53 mm, 2.65 μm film thickness respectively) from Agilent Technologies (Avondale, PH, USA). The detector and injector temperatures were at 300 and 220 °C, respectively. Helium was used as carrier gas at a constant flow rate of 7 ml/min. For FPD operation the hydrogen flow was set at 90 ml/min and the air one at 115 ml/min. Helium was used as the detector makeup gas at 30 ml/min. The temperature program of the GC oven was: initial *T* 50 °C (hold for 1 min), increased to 170 °C at 16 °C/min, ramped to 220 °C at the rate of 6 °C/min (hold for 1 min), increased to 240 °C at the rate of 4 °C/min, finally to 280 °C at the rate of 5 °C/min (hold for 10 min) and returned to initial conditions in 5 min. Total run time 40.8 min. The injection volume was 2 μl. The software for the control of the GC–FPD was ChromCard, ThermoFinnigan (Rodano, Milan, Italy).

Residue confirmation in real sample analysis were performed in a Trace 2000 GC equipped with a ThermoQuest autosampler (model AS2000), a split/splitless injector connected with the GCQ plus ion-trap mass spectrometer (Thermoquest, Austin, TX, USA), operating in either MS^n or SIM modes, injecting 2 μl of the tested solutions. The operation conditions of the GCQ Plus MS system were: the injector in splitless mode under isothermal conditions at 220 °C and the split valve was opened 1 min after the injection. Gas chromatography was carried out on DB-5MS (J&W Scientific) 0.25 μm, 30 m × 0.25 mm with a 1 m × 0.25 mm i.d. guard column of deactivated fused silica (Alltech, Augsburg, Germany). Oven temperature gradient was programmed as follows: the initial temperature was 50 °C for 1 min, and increased to 120 °C at the rate of 22.5 °C/min, ramped to 250 °C at 3 °C/min for 1 min and then increased to 285 °C at the rate of 15 °C/min which was held for 10 min and returned to the initial conditions in 5 min. Helium was the carrier gas at a flow rate of 1 ml/min. The MS system was operated in the electron impact ionization with positive polarity ion mode. The emission current was 250 mA, the multiplier voltage was 1700 V and a full scan range was set to 50–500 amu with maximum ion time 25 ms, 10 microscans and AGC target value of 50. The transfer line and the manifold temperature were set at 285 and 220 °C, respectively. Analytes were identified by comparing their EI mass spectra with home-made libraries.

Extraction procedures

MAE

Dry mate leaves (5 g) were weighed and put into the extraction vessels; 30 ml of acetonitrile (MeCN) were added in each

vessel and shaked vigorously by hand for 30 s. Sets of 12 vessels were microwave extracted according to the following operational parameters; magnetron power 800 W, maximum pressure 100 psi, heated to 80 °C in 10 min and maintained for 15 min.

After removing the vessels from the microwave oven, they were cooled at room temperature. The extract from each vessel was filtered under vacuum and rinsed with 15 ml MeCN. A 15 ml aliquot was transferred to a tube containing 1 ml toluene and evaporated until dryness under N_2 stream. Sample clean up consisted in two steps following a modification of the method described in 2003 by Haib et al. First, the dry extract was re-dissolved in 1 ml of MeCN and loaded into a 690 mg silica cartridge followed by the addition of 0.5 ml of toluene. The target compounds were eluted with 3 ml of an acetone–toluene (8:2) mixture. The 3 ml eluate was loaded into a 500 mg ENVI-carb cartridge and eluted with 3 ml of acetone. Each cartridge was pre-conditioned with 5 ml of acetone. The final eluate was collected, the solvent evaporated and the residue was dissolved in 200 μl of EtOAc for GC–FPD analysis.

QuEChERS

The employed procedure was a modification of the citrate buffered QuEChERS method CEN 15662 (www.cen.eu), (Payá et al., 2007; Anastassiades et al., 2010). A representative 2 g sample was weighed in a 50 ml PTFE centrifugation tube. Afterwards, 10 g of chopped ice and 10 ml of MeCN were added into each tube (Hayward et al., 2013; Rajski et al., 2013). Then 4 g of $MgSO_4$, 1 g of NaCl, 0.5 g of sodium citrate dibasic sesquihydrate and 1 g of sodium citrate tribasic dihydrate were added. The tube was hand shaken for 4 min and centrifuged, 10 min at 3000 × *g*. For the clean up step, a 6 ml aliquot of the extract was transferred to a 15 ml PTFE centrifugation tube containing 855 mg of $MgSO_4$, 150 mg of PSA and 45 mg of GCB. This tube was shaken for 20 s using a vortex and centrifuged for 10 min at 3000 × *g*. After that 40 μl of 5% formic acid in MeCN were added to 4 ml of extract and a 1 ml aliquot was transferred to a 5 ml conic tube and evaporated under nitrogen stream until dryness. Finally, the extract was dissolved in 200 μl of EtOAc for GC–FPD analysis.

Results and discussion

Extraction and clean up optimization

The analysis of pesticide residues using microwave assisted extraction systems require the optimization of different operational parameters such as magnetron power, temperature, pressure and extraction time. The optimum conditions for the extraction of pesticides by MAE in different matrices were selected taking into consideration previous reports (Niell et al., 2011; Vryzas et al., 2002, 2007; Papadakis et al., 2006; Vryzas and Papadopoulou-Mourkidou, 2002). QuEChERS and MAE protocols yielded highly pigmented extracts and GCB was used in the clean up step to remove the co-extracted chlorophyll. However, the amount of GCB used was a balance between the recoveries of the studied pesticides and the pigment removal. In the MAE protocol, an ENVICARB cartridge was used, according to the method proposed by Hayward et al. for herbs, whereas QuEChERS used GCB and PSA in a dispersive mode. Nevertheless, PSA was not employed in MAE method, as polyphenols and shikimic acid analogs such as chlorogenic acid present in *I. paraguaiensis* could be analyte protectants for the most labile pesticides by interacting with the silvnol free OH in the glass liner as it has been established in the literature (Anastassiades et al., 2003).

Methods performance and validation

All validation procedures were performed using a commercial yerba mate sample labeled as organic, which was previously analyzed in order to determine the pre-existent pesticide residues content.

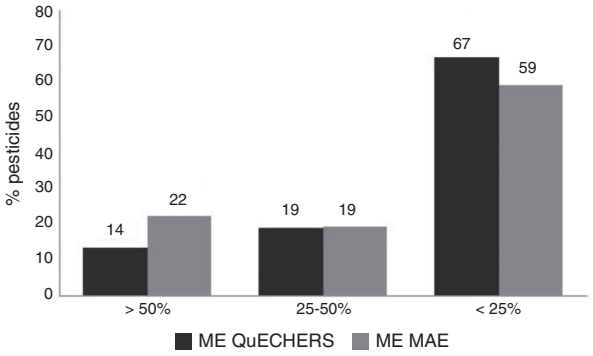

Fig. 1. Calculated matrix effects of MAE and QuEChERS method. Matrix effect (%) = (1 − (slope matrix/slope solvent)) × 100.

The method efficiency, expressed as recovery rates and relative standard deviation (% RSD) of the tested pesticides, was determined at two fortifications levels: 0.2 and 0.5 mg/kg in spiked samples of yerba mate, as it is shown in Table 1.

Among the 42 pesticides included in the analytical method phorate, fenthion, terbufos, fenamiphos, and metamidofos exhibit recoveries lower than 50% for both methods and cannot be determined according to DG-SANCO guidelines (European Commission DG-SANCO, 2014). The remaining analytes presented differences in the recovery results for both methods. Particularly with MAE extraction, fensulfothion was not detected at any fortification level, while dichlorvos, phosphamidon and dimefox presented recoveries between 19 and 63% at 0.2 mg/kg. QuEChERS method presented low recoveries for omethoate at 0.2 mg/kg, prothiofos at both levels and chlorpyrifos presented recoveries of 65 and 59% at 0.2 and 0.5 mg/kg respectively.

These low recoveries could be due to the possible volatilization or degradation during GC determination (Ingelse et al., 2001) or due to the losses during the concentration process. It was observed that most of the pesticides showing low recoveries are volatile and have the smallest retention times (Table 1). Concerning the QuEChERS method most of the pesticides with low recoveries eluted in the middle of the chromatogram and after caffeine.

As it is shown in Fig. 1, QuEChERS method showed lower matrix effect than MAE. Signal enhancement was observed for 41 and 33% of the studied pesticides in MAE and QuEChERS, respectively. Particularly mevinphos showed 75% of signal enhancement in QuEChERS method, this could lead to over quantification, as pointed out by the DG-SANCO guidelines, explaining the high recovery observed.

Matrix-matched calibration curves were linear in the range 0.05–4 μg/ml with correlation coefficients (r^2) higher than 0.99 in most cases. Only dichlorvos presented linearity problems in QuEChERS and this could be attributed to its high volatility and thermal lability. These problems were not observed in MAE, supporting the hypothesis of the analyte protectant effect of mate polyphenols.

The limits of detection (LOD), ranged from 0.004 to 1 mg/kg. The LOQ, determined as established in DG-SANCO guidelines is the lowest concentration of the analyte that has been validated with acceptable accuracy by applying the complete analytical method, ranged from 0.1 to 0.2 mg/kg for most of the evaluated pesticides. However, considering the LOQ as the LOD × 10, 28/33 pesticides presented a LOQ below 0.2 mg/kg in MAE and 11/27 in QuEChERS. Some pesticides such as phenthoate, prothiofos, parathion ethyl, omethoate, dimefox and chlorpyrifos in QuEChERS method and dichlorvos, dimefox, fensulfothion and phosphamidon in MAE

Table 1
(%) Recovery rates and respective RSD obtained for MAE and QuEChERS method at 0.2 and 0.5 mg/kg spiking levels (pesticides with acceptable recoveries to one at least of the tested methods were only included).

Pesticide common name	Stock mix	RT (min)	Spiking level (mg/kg)	MAE		QuEChERS	
				Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Acephate	I	11.59	0.2	84	4	70	4
			0.5	92	3	77	9
Bromophos methyl	III	21.98	0.2	97	11	75	11
			0.5	93	6	65	11
Cadusafos	III	15.85	0.2	84	11	99	4
			0.5	80	4	91	9
Chlorfenvinphos	I	22.68	0.2	89	5	93	4
			0.5	94	3	74	12
Chlorpyrifos	III	21.19	0.2	86	11	65	4
			0.5	82	5	59	13
Chlorpyrifos methyl	II	19.29	0.2	90	6	76	8
			0.5	89	10	73	6
Diazinon	I	17.48	0.2	91	1	77	4
			0.5	88	4	76	15
Dichlorvos	II	9.55	0.2	63	16	99	4
			0.5	67	15	109	10
Dimefox	II	7.38	0.2	50	15	85	6
			0.5	53	14	113	14
Dimethoate	II	16.13	0.2	112	6	96	8
			0.5	109	3	107	14
Disulfoton sulfoxide	III	10.50	0.2	91	9	117	12
			0.5	90	4	117	9
Disulfoton sulfone	I	23.47	0.2	105	2	119	5
			0.5	110	1	95	21
Ethion	III	26.64	0.2	103	9	76	3
			0.5	101	4	65	16
Ethoprophos	II	14.91	0.2	100	6	78	6
			0.5	101	4	90	9
Fenchlorphos	II	19.96	0.2	95	5	70	4
			0.5	91	9	67	6
Fenitrothion	III	19.99	0.2	118	11	99	5
			0.5	111	3	91	13
Fonofos	III	17.26	0.2	86	12	85	4
			0.5	80	4	76	11
Fensulfothion	II	26.67	0.2	ND	ND	82	17
			0.5	ND	ND	89	9
Heptenophos	III	13.78	0.2	90	12	119	4
			0.5	85	5	116	7
Malathion	II	20.52	0.2	104	3	80	3
			0.5	99	8	88	9
Mecarbam	III	22.49	0.2	100	10	102	4
			0.5	97	3	93	12
Methidathion	II	23.28	0.2	107	7	102	10
			0.5	103	9	98	7
Mevinphos	III	11.69	0.2	88	13	131	3
			0.5	83	6	134	4
Omethoate	II	13.98	0.2	93	12	49	18
			0.5	79	16	81	25
Parathion ethyl	II	21.16	0.2	103	4	61	5
			0.5	96	9	74	9
Parathion methyl	III	19.14	0.2	118	12	116	3
			0.5	117	1	100	11
Phenthoate	II	22.76	0.2	109	3	66	3
			0.5	101	8	76	8
Phosphamidon	I	6.24	0.2	19	12	87	7
			0.5	22	10	80	8
Pirimiphos methyl	I	20.30	0.2	100	18	74	6
			0.5	91	4	64	14
Profenofos	III	24.74	0.2	118	13	88	7
			0.5	113	4	77	14
Prothiofos	II	24.83	0.2	99	5	43	7
			0.5	94	9	50	5
Quinalphos	III	22.70	0.2	89	11	93	5
			0.5	86	6	85	12
Terbufos sulfone	II	22.30	0.2	106	3	88	2
			0.5	101	8	86	11
Thionazin	II	14.41	0.2	97	7	78	6
			0.5	96	4	94	10
Tolclofos methyl	I	19.50	0.2	88	2	75	4
			0.5	89	3	64	12
Triazophos	I	26.73	0.2	98	6	114	3
			0.5	104	3	80	14
Trichlorfon	I	5.05	0.2	90	1	100	6
			0.5	89	4	92	12

Table 2
Limits of detection (LOD) and limits of quantification (LOQ) in mg/kg in GC/FPD.

Pesticide common name	MAE mg/kg		QuEChERS mg/kg		MRL (EU) mg/kg
	LOD	LOD × 10/LOQ	LOD	(LOD × 10/LOQ	
1. Acephate	0.01	0.1/0.2	0.05	0.5/0.2	0.05
2. Bromophos ethyl	0.004	0.04/0.2	0.05	0.5/0.2	0.1
3. Cadusafos	0.004	0.04/0.2	0.01	0.1/0.2	0.01
4. Chlorfenvinphos	0.01	0.1/0.2	0.05	0.5/0.2	0.05
5. Chlorpyrifos	0.004	0.04/0.2	0.1	1.0/1.0	0.5
6. Chlorpyrifos methyl	0.004	0.04/0.2	0.05	0.5/0.2	0.1
7. Diazinon	0.004	0.04/0.2	0.01	0.1/0.2	0.05
8. Dichlorvos	0.01	0.1/0.5	0.05	0.5/0.2	0.02
9. Dimefox	0.05	0.5/1.0	0.05	0.5/0.2	0.01
10. Dimethoate	0.05	0.5/0.2	0.05	0.5/0.2	0.1
11. Disulfoton sulfoxide	0.01	0.1/0.2	0.05	0.5/0.2	0.05
12. Disulfoton sulfone	0.004	0.04/0.2	0.01	0.1/0.2	
13. Ethion	0.004	0.04/0.2	0.05	0.5/0.2	0.05
14. Ethoprophos	0.004	0.04/0.2	0.01	0.1/0.2	0.02
15. Fenchlorphos	0.05	0.5/0.2	0.05	0.5/0.2	0.1
16. Fenitrothion	0.01	0.1/0.2	0.05	0.5/0.2	0.05
17. Fonofos	0.004	0.04/0.2	0.01	0.1/0.2	0.01
18. Fensulfothion	1.0	1.0/1.0	0.05	0.5/0.2	0.01
19. Heptenophos	0.004	0.04/0.2	0.01	0.1/0.2	0.01
20. Malathion	0.05	0.5/0.2	0.05	0.5/0.2	0.02
21. Mecarbam	0.01	0.1/0.2	0.05	0.5/0.2	0.1
22. Methidathion	0.004	0.04/0.2	0.05	0.5/0.2	0.1
23. Mevinphos	0.004	0.04/0.2	0.01	0.1/0.2	0.02
24. Omethoate	0.01	0.1/0.2	0.05	0.5/0.5	0.05
25. Parathion ethyl	0.01	0.1/0.2	0.05	0.5/0.5	0.1
26. Parathion methyl	0.004	0.04/0.2	0.01	0.1/0.2	0.05
27. Phenthoate	0.01	0.1/0.2	0.05	0.5/0.5	0.01
28. Phosphamidon	0.01	0.1/1.0	0.05	0.5/0.2	0.02
29. Pirimiphos methyl	0.004	0.04/0.2	0.01	0.1/0.2	0.3
30. Profenofos	0.01	0.1/0.2	0.05	0.5/0.2	0.1
31. Prothiofos	0.01	0.1/0.2	0.05	0.5/1.0	0.01
32. Quinalphos	0.004	0.04/0.2	0.01	0.1/0.2	0.1
33. Terbufos sulfone	0.004	0.04/0.2	0.01	0.1/0.2	0.01
34. Thionazin	0.004	0.04/0.2	0.01	0.1/0.2	0.01
35. Tolclofos methyl	0.05	0.5/0.2	0.05	0.5/0.2	0.1
36. Triazophos	0.004	0.04/0.2	0.05	0.5/0.2	0.02
37. Trichlorfon	0.004	0.04/0.2	0.01	0.1/0.2	0.05

method showed LOQ higher than 0.2 mg/kg. as they could not be validated with acceptable accuracy at this level (Table 2).

Chromatographic analysis

Two megabore columns in tandem were used in order to achieve adequate chromatographic separation. Megabore columns (typically 10 m × 0.53 mm) are advantageous compared to narrow- or micro-bore columns when extracts of "difficult" matrices have to be analyzed since megabore columns can provide high loadability as films up to 5 μm (Cajka et al., 2008; Ravindra et al., 2008.). The use of two megabore columns in tandem (20 m × 0.53 mm × 2.65 μm) can also improve the chromatographic separation of pesticides with similar properties, a key point when the GC is not connected with a MS detector (Mastovska and Lehotay, 2003). Therefore, the selection of a column with high internal diameter (0.53 mm) and film thickness (2.65 μm) ensure better performance in samples with high matrix effect. A long oven temperature gradient was selected (run time 40.8 min) to improve the chromatographic resolution of the analytes which are difficult to resolve under typical GC conditions. The OP pesticides included in the analytical method were separated in three stock solutions based on the retention time of each analyte (Table 1). Separation of target compounds was performed in order to avoid co-elution of some pesticides. Fig. 2 shows the chromatogram obtained for the analysis of fortified yerba mate samples with Mix I at 0.1 mg/kg with both MAE and QuEChERS methods by GC–FPD.

As it is presented in the chromatograms (Fig. 2), there is a peak with retention time around 20 min corresponding to caffeine. The clean up of both methods is not enough to remove all the caffeine, although MAE clean up is more efficient than QuEChERS.

Real sample analysis

In order to check the performance of the method nine commercial samples were analyzed. The samples were extracted using both validated methods and analyzed by GC/FPD and the positive findings were confirmed by GC/MS.

Acephate, ethoprophos, chlorpyrifos, and cadusafos were detected in commercial samples and their concentrations are shown in Table 3. However, only chlorpyrifos showed concentrations above the LOQ of MAE method in five samples and below the corresponding MRL (European Commission, 2005, 2014).

MAE and QuEChERS comparison

The analytical results of real samples shown in Table 3 indicate that, under the experimental conditions employed in the present communication, MAE provides better extractability of incurred residues present in real samples as it detects not only more pesticides but also the residue concentrations found are higher than QuEChERS.

The reason of these results could be based in the efficiency of microwave energy, which is higher than manual agitation for the extraction of the residues from the matrix.

Concerning matrix effect MAE presented more compounds showing signal enhancement than QuEChERS (Fig. 1). However, is

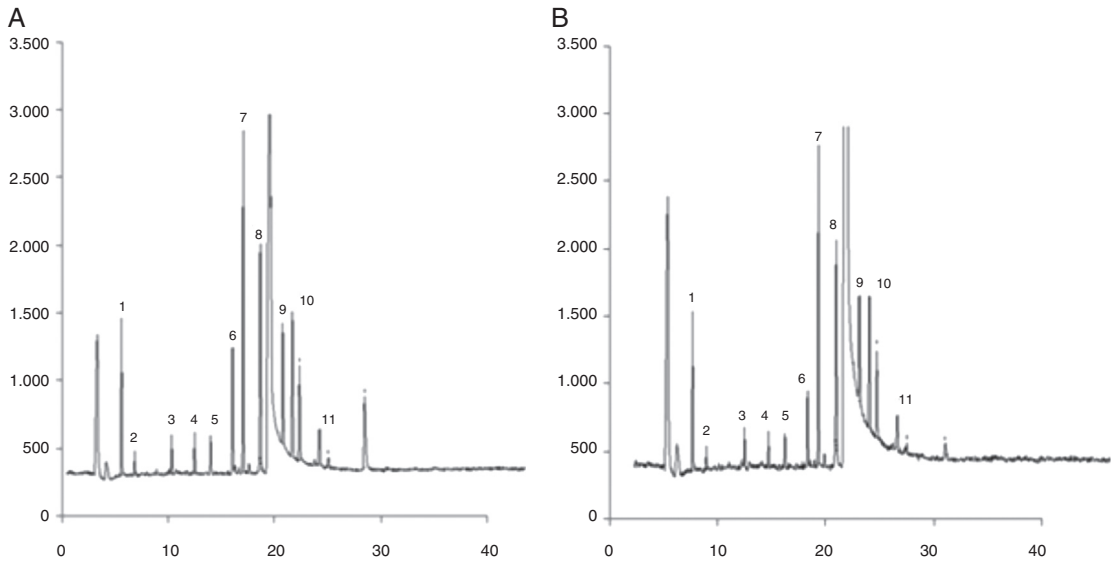

Fig. 2. Chromatograms of fortified mate samples with Mix I at 0.1 mg/kg by GC–FPD. MAE (A) and QuEChERS (B) methods. 1: trichlorfon; 2: phosphamidon; 3: acephate; 4: phorate; 5: diazinon; 6: tolclofos methyl; 7: pirimiphos methyl; 8: fenthion; 9: chlorfenvinphos; 10: disulfuton sulfone; 11: triazophos.

Table 3
Pesticides (mg/kg) detected by GC–FPD and confirmed by GC–MS in real samples. ND: not detected.

Real sample	Acephate MAE/QuEChERS	Ethoprophos MAE/QuEChERS	Chlorpyrifos MAE/QuEChERS	Cadusafos MAE/QuEChERS
1	<LOQ/ND	<LOQ/ND	ND	ND
2	ND	ND	0.3/<LOQ	ND
3	ND	ND	<LOQ/ND	ND
4	ND	ND	ND	ND
5	ND	ND	<LOQ/ND	ND
6	ND	ND	0.2/<LOQ	ND
7	ND	ND	0.4/<LOQ	ND
8	ND	ND	0.2/ND	<LOQ/ND
9	ND	ND	0.2/ND	<LOQ/ND

more effective avoiding the extraction of caffeine, which is the main detected interference.

In general, MAE method ensured lower to similar LOD for all pesticides except for fensulfothion, compared with QuEChERS method, while the LOQ (lowest validated level) for 28 pesticides in both methods were similar. If LOQ are calculated as LOD × 10, 13 pesticides could be assessed for MRL compliance with MAE method and three pesticides with QuEChERS.

Comparing the accuracy and precision of both methods, MAE presented better performance than QuEChERS, since the recoveries of 33 pesticides were within the range 70–118% with RSDs from 1 to 18%. QuEChERS method presented recovery rates between 70 and 120% and RSDs in the range 3–21% for 27 pesticides, at the lowest spiking level. Some of the obtained results in this study with QuEChERS method were similar to those reported by Lozano et al. (2012), in different types of tea using GC-QqQ/MS.

QuEChERS methodology is simple, cheap, practically no glassware is needed, and it is more environmentally friendly as the solvent consumption is lower than MAE.

MAE presented good performance, it allows the simultaneous extraction of 10 samples, but the equipment required is not often available in the laboratories.

The present study demonstrated that although both methods are suitable for the analysis of pesticide residues in yerba mate, MAE presented a better performance under the experimental conditions tested.

Yerba mate is consumed daily by almost 50 million people but there are few data on the literature concerning the persistence of pesticide residues in the processed leaves. This work might help to gather the information needed to perform studies on pesticide residue exposure of the population due to yerba mate intake.

Authors' contributions

JG, SN and LP performed the laboratory work, data and chromatographic analysis. HH and ZV ran the first trial experiments with MAE. ZV analyzed the real samples in the GC–(ITD)-MS, LP, ZV and SN drafted the paper. VC, LP and HH gave the works conceptual frame, participated in the results discussion and the manuscript final writing. ZV and EPM supervised the laboratory work and ZV contributed to critical reading of the manuscript.

Conflict of interest

The authors have no conflict of interest to declare.

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