Talanta 81 (2010) 385–391

Contents lists available at ScienceDirect

Talanta

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

Simplified QuEChERS approach for the extraction of chlorinated compounds from soil samples

Carmelo García Pinto, María Esther Fernández Laespada, Sara Herrero Martín, Ana María Casas Ferreira, José Luis Pérez Pavón*, Bernardo Moreno Cordero

Departamento de Química Analítica, Nutrición y Bromatología, Facultad de Ciencias Químicas, Universidad de Salamanca, 37008 Salamanca, Spain

ARTICLE INFO

Article history:
Received 9 September 2009
Received in revised form 3 December 2009
Accepted 10 December 2009
Available online 16 December 2009

Keywords:
Simplified QuEChERS approach
Chlorinated compounds
Soil samples

ABSTRACT

A simplified version of the QuEChERS method for the extraction of chlorinated pollutant compounds from soil samples is proposed. The procedure involves simple liquid extraction of the soil sample with ethyl acetate, followed by the addition of anhydrous $MgSO_4$. Gas chromatography/electron capture detection (ECD) is then used to analyse the extracts without any other sample pretreatment. This new QuEChERS version includes, therefore, fewer treatment stages of the sample, which makes the final procedure simpler, faster, and cheaper and minimizes the creation of errors associated with this step. Three chlorinated compounds (chloroform, 1,2-dichlorobenzene, and hexachlorobenzene) of different volatility and polarity have been selected as target compounds and two different solvents (acetonitrile and ethyl acetate) have been evaluated in order to prove the suitability of the proposed approach for the extraction of these compounds from different soil samples. The suitability of the acetonitrile and ethyl acetate for PTV-GC analysis has also been evaluated. Recoveries between 62 and 93% and reproducibilities between 3.5 and 7.6% have been achieved.

1. Introduction

Determination of organic volatile/semivolatile compounds in environmental samples, such as air, water, soil or sediments usually requires special pretreatment prior to the final determination, most often performed by gas chromatography. This pretreatment involves the isolation from the matrix of the compounds of interest and their transfer to other medium, ideally with the simultaneous removal of interfering substances and selective enrichment in the receiving medium to a concentration higher than the detection limit of the proposed procedure [1].

The choice of sample treatment applied depends heavily on the complexity of the matrix. Water, in general, represents a less complicated matrix than air, sediment or soil samples. This choice is also related to the detection method. The more sensitive and specific detection method is used, the less stages of sample treatment will be required [2]. Modern analytical strategies tend towards automatization and integration of sample pretreatment in the chromatographic systems as far as possible [3].

Development of solventless (or at least with low solvent consumption) sample preparation techniques constitutes a pillar of green analytical chemistry [4] and has taken a rapid development during last years. The great interest in this approach is due to toxicological, environmental and economical aspects. A number of techniques with those characteristics have been developed [5,6] such as single drop microextraction (SDME), liquid phase microextraction (LPME), solid phase microextraction (SPME) and stir-bar sorptive extraction (SBSE). Among techniques based in gas extraction, static headspace (SHS), purge and trap (P&T) and closed loop stripping analysis (CLSA) could be mentioned. Membrane extraction approaches such as membrane assisted solvent extraction (MASE), membrane extraction with sorbent interface (MESI) or membrane inlet mass spectrometry (MIMS) have also been applied to environmental samples [7].

QuEChERS (quick, easy, cheap, effective, rugged and safe) procedure was introduced by Anastassiades in 2003 as a new approach to extract a wide range of pesticides from different food matrices with high water content [8]. This basic procedure is based on a liquid partitioning with acetonitrile followed by a dispersive SPE clean-up with primary secondary amine (PSA). Modifications to the original method to ensure efficient extraction of pH dependent compounds (by using different buffers solutions) [9–12] or addition of water to dry samples in order to obtain the necessary moisture [13–15] have been introduced.

To remove matrix components in the clean-up step, modifications of the original dispersive SPE step by using graphitized carbon black (GCB) and C_{18} sorbent [10], SPE in cartridge [16] or Florisil cartridges [17,18] have been used. The QuEChERS method

* Corresponding author. Fax: +34 923 294483.
E-mail address: jlpp@usal.es (J.L.P. Pavón).

0039-9140/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2009.12.013

is particularly popular for determination of polar, middle polar and non-polar pesticide residues in various food matrices [19–26] because of its simplicity, inexpensiveness, amenability to high throughput, and relatively high efficiency results with a minimal number of steps. Recently, the QuEChERS method for multiple residue pesticides in fruits and vegetables has received the distinction of Official method of AOAC International [27].

Although QuEChERS has mainly been used for the determination of pesticides, some other compounds, such as pharmaceuticals [28], β-lactam antibiotics [29,30] or veterinary drugs [30–34] have been determined using QuEChERS. To the best of our knowledge the use of QuEChERS in soils is very limited [35] but with very good results. In the above-mentioned report, the clean-up step of the extracts was carried out by dispersive SPE. According to these experiences, the development of new applications and modifications of the method is of great interest.

In this paper, a new and simplified version of the QuEChERS method is proposed for the extraction of chlorinated pollutant compounds from soil samples. To solve the main disadvantage associated to the QuEChERS methodology (low preconcentration of the compounds in the extracts), analysis by gas chromatography with a micro-electron capture detector (μECD), which improves the selectivity and sensitivity with respect to conventional detectors, is proposed.

The main advantage of the proposed version is related to the elimination of the dispersive SPE step after the extraction. This step has demonstrated to be highly effective to reduce lipid matrix co-extractives from the extracts, and it is faster, cheaper and easier than traditional SPE clean-up procedures. However, due to the non-fatty characteristics of the soil matrices and the high degree of selectivity and sensitivity of the GC–μECD system, it was decided to analyse the extracts, obtained after the centrifugation step, without conducting further clean-up. In consequence, the new QuEChERS version includes fewer treatment stages of the sample, which makes the final procedure simpler, faster, and cheaper and minimizes the errors associated with this step.

In order to prove the suitability of the proposed approach, chlorinated compounds of different characteristics related to their volatility and polarity have been chosen. These analytes are very important organic pollutants, because of their common use and high toxicity. The International Agency for Research on Cancer (IARC) has classified the three target compounds as possibly carcinogenic to humans (Group 2B), based on limited evidence of carcinogenity in humans but sufficient evidence of this in experimental animals. Two solvents (acetonitrile and ethyl acetate) have been evaluated in terms of their suitability for chromatographic analysis and of their extraction efficiency from different soil matrices.

2. Experimental

2.1. Chemicals

Chloroform (99.9% purity) was supplied by Supelco (Bellefonte, PA, USA) and 1,2-dichlorobenzene (99% purity), and hexachlorobenzene (99% purity) were from Sigma–Aldrich (Steinheim, Germany). Acetonitrile (MeCN) was from Merck (Darmstadt, Germany) and ethyl acetate (EtOAc) from Sigma–Aldrich. Magnesium sulfate anhydrous and sodium chloride were from Scharlau (Barcelona, Spain). Ultrapure quality water obtained with an Elgastat UHQ water purification system was used.

2.2. Standard solutions

Stock solutions (500 mg/L in ethyl acetate or acetonitrile) of each compound were prepared and stored in a refrigerator at 4 °C. From these, different solutions were prepared by dilution in each of the solvents. They were used in the studies of the different modes of injection, as well as in the spiking of soils at the required concentration levels.

2.3. Soil samples

Three different types of soils were used to evaluate the proposed QuEChERS methodology. Two collected soils: a garden soil, with high organic content (Salamanca, Spain), and a Vertisol, which has a high percentage of clay (Tabasco, Mexico), as well as a certified reference material RTC-CRM631 (silty clay soil) with certified content for chloroform purchased from LGC Promochem (Barcelona, Spain). The absorption capacity of soils is strongly governed by their contents in sand, clay and organic matter. Therefore, the soils studied are extreme examples of soil types, and the results obtained could be extrapolated to most natural soils.

In order to avoid the presence of any of the compounds studied in the soils, collected samples (garden soil and Vertisol) were air-dried on a heating plate at 90 °C for 48 h, with frequent turning. This procedure removed any organic traces or humidity from the soil. These soil blanks were checked to be free of the target analytes before spiking.

The spiking procedure was as follows: 20 g of soil was placed in a 100 mL amber flask and 2 mL of the target analytes solution (at suitable concentrations) in ethyl acetate was added. The flask was hermetically sealed and shaken vigorously for 15 min to achieve perfect homogenization of the compounds in the matrix. To allow the interaction between the compounds and the matrix the samples were stored in a refrigerator at 4 °C for 15 days.

2.4. Apparatus

Gas chromatographic analysis was performed with an Agilent 7890A chromatograph equipped with a ^{63}Ni micro-electron-capture detector (μECD). According to the specifications, the detection zone volume of this detector is 10 times smaller than any other ECD, which translates into greater sensitivity and decreases the chance of cell contamination. A DB-VRX capillary column (20 m × 0.18 mm × 1 μm) for fast gas chromatography from Agilent J&W was used. The carrier gas was helium N50 (99.995% pure; Air Liquide).

All experiments were carried out with an Agilent 6890 PTV inlet. The PTV was equipped with a 71 mm × 2 mm liner (internal volume of 180 μL) packed with Tenax-TA, a hydrophobic polymer designed to trap organics. The sample was introduced through an automatic liquid sample injection system (Agilent 7683).

2.5. Analytical procedure

For sample pretreatment with the simplified QuEChERS approach, 2.5 g of soil sample was weighed in a 15 mL glass centrifuge tube with screw cap, which keeps the tube closed for most of the process of sample preparation, thus avoiding as much as possible losses of volatile compounds during this stage. 1.5 mL of ultrapure water was added on the soil sample in order to make pores in the sample more accessible to the extraction solvent and to homogenize water content in different soil samples and the mixture was shaken for 1 min with a Vortex device. Then, 2.5 mL of ethyl acetate (extraction solvent) was added and the mixture was shaken again during 1 min. Following this, 1 g of magnesium sulfate was added, shaking it for 1 min as quick as possible to prevent formation of $MgSO_4$ conglomerates. The tube was centrifuged at 5000 rpm during 5 min. A comparison between the original QuEChERS approach and the modifications proposed in this paper is summarized in Fig. 1.

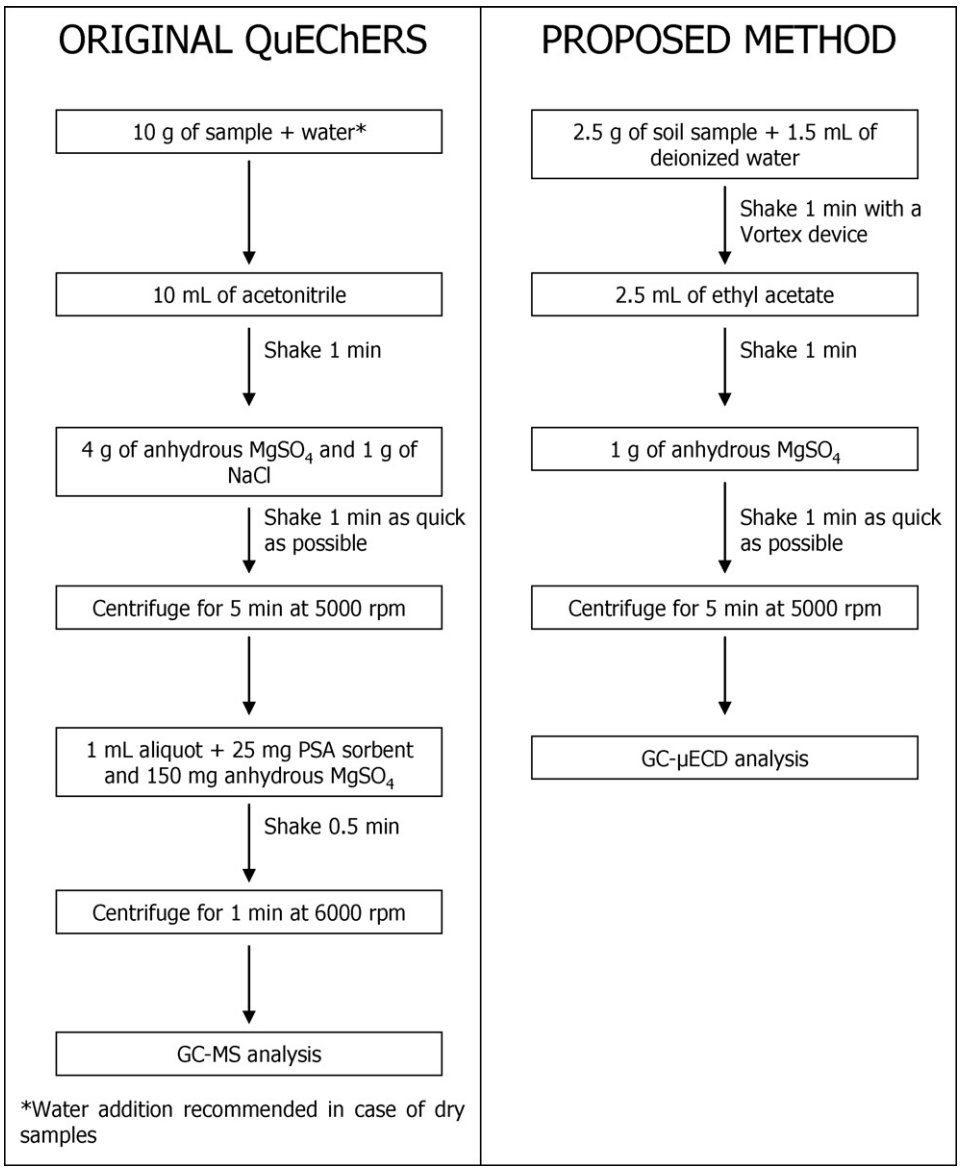

Fig. 1. Comparison of the proposed method with the original QuEChERS adapted to dry samples.

The analysis of the extracts was performed by a GC provided with a μECD. Two injection modes were used: splitless injection for the volatile compound (chloroform) and solvent vent injection for semivolatile compounds (1,2-dichlorobenzene and hexachlorobenzene).

In the splitless injection, 0.2 μL of sample was injected and the injector temperature was kept at 250 °C throughout the analysis time. The splitless time was 1 min. In the solvent vent mode, the injector starting temperature was 30 °C. The injection volume was 5.0 μL. The vent flow was adjusted to 20 mL/min and the vent pressure to 5.00 psi. After 0.5 min, the split valve was closed and the liner was flash-heated at 12 °C/s to 300 °C. The analytes were transferred from the liner to the capillary column (1.5 min injection time). The split valve was then opened and the liner temperature was held at 300 °C for 5.00 min to allow the cleaning of the liner thus avoiding the possibility of memory effects. In both cases, the septum purge flow was 4.0 mL/min.

The column oven temperature involved an initial temperature of 60 °C for 2 min, this was increased at 65 °C/min to 175 °C, and then further increased at 45 °C/min to 240 °C and held for 3.05 min. The latter two temperature ramps are the maximum ones permitted by the instrumental configuration employed. The carrier gas was He and the flow was 1.4 mL/min. The total chromatographic run time was 8.26 min.

The μECD parameters were a detection temperature of 300 °C and a make up flow gas (N_2) of 20 mL/min.

3. Results and discussion

3.1. Selection of solvent for PTV-GC analysis

The solvents most commonly used for multiresidue analysis of pesticides have been MeCN, acetone and EtOAc; each of them gives acceptably high recoveries for a wide range of pesticides in different food matrices [8].

Regarding the suitability of the organic solvents for gas chromatography, Mastovská and Lehothay [11] evaluated and compared the possibilities of MeCN, acetone, and EtOAc. The three solvents can directly serve as a medium for GC injection and therefore solvent exchange is not required before the chromatographic analysis.

Table 1
Characteristics of the compounds under study.

Compounds	Boiling point (°C)	Log K_{ow}	K_{oc} (L/kg)
CFM	62	1.97	40
1,2-DCB	180–183	3.38	617
HCB	323–326	6.2	54954

Soil samples, in contrast with fruits and vegetables, do not have high contents of lipid materials. Different soil types are characterised by their mineral fraction (variable percentages of sand, silt and clay) and organic matter fraction (10–15%) mainly composed by humic substances Therefore, the main disadvantage of EtOAc (co-extraction of non-polar compounds such as lipids or waxes) may not be significant here, and any of the three organic solvents could be suitable for the extraction and chromatographic determination of chlorinated compounds from soil matrices. In this work, acetone was not investigated as extraction solvent, due to its disadvantages in phase separation and to its high volatility. Therefore, MeCN and EtOAc were evaluated in relation with their chromatographic behaviour.

In order to evaluate the possibilities of the new proposed approach a set of organic chlorinated compounds with very different properties related to volatility, polarity and their interaction with soil was selected. The set of target compounds includes: a volatile compound, chloroform (CFM) and two semivolatile compounds, 1,2-dichlorobenzene (1,2-DCB) and hexachlorobenzene (HCB). The volatility (expressed as their boiling point), the polarity (expressed as the value of their log $K_{o/w}$), and the interaction with soil (expressed as the value of their organic carbon partition constant K_{oc}) for these compounds are shown in Table 1.

According to the differences in the properties of the target analytes, and in order to achieve optimal analytical signals for every compound, it was decided to study separately the volatile organic compound from the semivolatile ones, because they could be influenced in a very different way by solvent and conditions used for injection.

Firstly, 500 μg/L solutions of chloroform were prepared in MeCN and EtOAc and injected in the gas chromatograph with three different injection modes allowed by the programmable temperature vaporizer used: hot split, hot splitless and solvent vent. Fig. 2 shows the chromatograms obtained. When hot split injection mode was used it was observed that chromatographic resolution obtained with EtOAc was better than with MeCN; the peak of CFM was narrower and therefore better signal to noise ratio was obtained. However, the main disadvantage of split injection is that most of the sample is wasted through the split line, and therefore it is not the most appropriate technique for trace analysis, that requires maximum sensitivity.

When the solution of CFM in EtOAc was injected in hot splitless mode, a significant improvement in peak area and height was achieved on comparing with split injection. Nevertheless, when the same injection mode was used for the MeCN solution a clear distortion of peak shape was obtained. This poor resolution can be explained by the high expansion volume of MeCN (506 μL) which generates a larger vapour volume and analyte residence time in the injection port than ethyl acetate (272 μL). With this injection mode, most of the solvent is probably focused to the column inlet in the splitless injections, causing problems with the peak shapes. Other authors also suggest that in the splitless injection mode solvents with less volume expansion are preferred [11].

When the two solutions of CFM in EtOAc and MeCN were injected using the solvent vent mode (injection volume 0.2 μL, initial temperature 5 °C, purge time 1 min, purge flow 50 mL/min, injection time: 1.5 min) the same effect of worse chromatographic resolution and wider peak with MeCN, observed for the other two

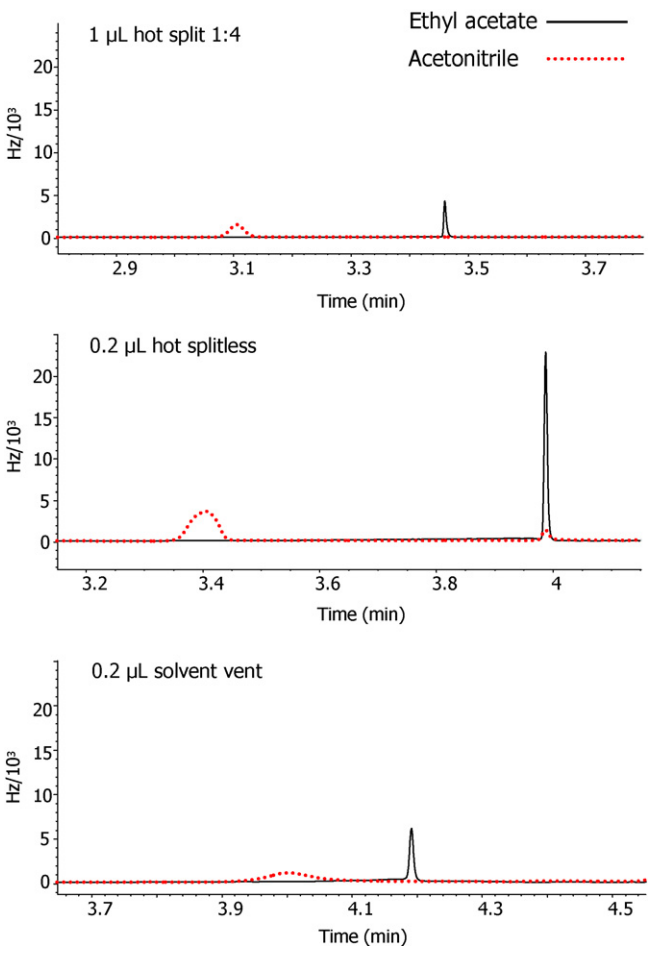

Fig. 2. Different injection modes for chloroform in acetonitrile and ethyl acetate.

injection modes studied, was obtained. On comparing this mode with the hot splitless mode it was noticed that losses of the compounds occurred when the same amount of sample was injected in solvent vent mode. Moreover, worse peak reproducibility between injections was obtained. These results could easily be explained by the volatility of the compound (boiling point of 61 °C) which is lower than the boiling points of the solvents (77 and 82 °C for EtOAc and MeCN, respectively). The use of a liner packed with Tenax-TA® did not solve this problem, due to the retention of the solvents investigated at low temperatures. Nevertheless none of the commercially available packing materials (glass wool, carbotrap C, carbotrap B) showed better properties for the combination of solvents and analyte studied here.

In the case of semivolatile compounds, the use of a programmed temperature vaporizer (PTV) inlet offers an interesting alternative for increasing sensitivity with the solvent vent injection mode. The boiling point of the solvents are sufficiently low and, therefore, adequate to trap the analytes in the liner at acceptable high venting temperatures (to avoid a need for excessive cooling), but more importantly, to be able to eliminate the majority of the solvent by venting without losing the analytes. Additionally, this allows the injection of large sample volumes, with a consequent increase in sensitivity.

Fig. 3 shows the chromatograms obtained for the two chosen semivolatile compounds (1,2-dichlorobenzene at 250 μg/L and hexachlorobenzene at 50 μg/L) when increasingly larger acetonitrile and ethyl acetate solution volumes are injected. It is clear that, for acetonitrile, a strong distortion of the chromatographic

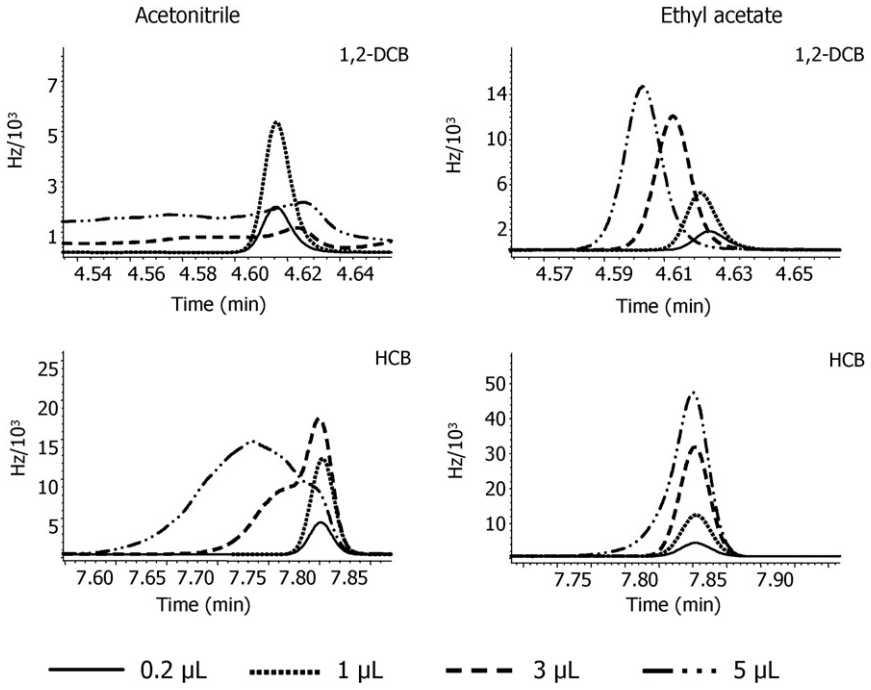

Fig. 3. GC/ECD chromatograms obtained by injecting (solvent vent injection mode) different volumes of semivolatile compound solutions in acetonitrile and ethyl acetate.

peaks occurs at large injection volumes, making impossible to work with injection volumes greater than 1.0 μL. This behaviour, as seen with the volatile compound, can be explained by the large volume expansion of acetonitrile. With solvent vent injection, the effect is observed at volumes larger than 1.0 μL, instead of 0.2 μL because, in the first place, this injection mode allows the removal of most of the solvent during the venting step, and, in the second place, the semivolatile compounds elute when the chromatographic column temperature is high, and therefore when the solvent has been completely eluted, thus avoiding the distortion caused by it.

Regarding ethyl acetate, on increasing the injection volume the chromatographic signal accordingly increases, thus providing better sensitivity. In this case, the boiling point of the solvent, slightly lower than that of acetonitrile, makes it possible a more effective solvent elimination during the venting process, thus allowing the injection of volumes up to 5 μL, without distortion of the chromatographic peaks. The difference in signals between 3 and 5 μL, allows to predict that injection of larger sample volumes would not improve the results significantly.

Therefore, from a chromatographic point of view, ethyl acetate presented advantages in terms of chromatographic resolution for the compounds studied and allowed larger injection volumes using hot splitless injection mode for chloroform (0.2 μL in the optimized experimental conditions) and solvent vent injection mode for semivolatile compounds (5 μL in the optimized experimental conditions), which gives rise to improved sensitivity of the chromatographic methodology.

Nevertheless, in case that acetonitrile would be used as solvent, the optimal injection conditions would imply hot split for chloroform (1.0 μL, split ratio 1:4), and hot splitless for the semivolatile compounds (1.0 μL).

3.2. Simplified QuEChERS approach applied to soil samples

Once the solvents had been compared in terms of their chromatographic resolution and analyte response, a study to determine the different parameters involved in the extraction efficiency in soil samples was done.

The extraction method used for this experience, followed the main steps and proportions of the original QuEChERS method (except for the extract clean-up): 2.5 g of spiked sample were homogenized with 1.5 mL of water using vortex mixing and then 2.5 mL of solvent were added and the sample was shaken with a vortex device again and, after that, a combination of anhydrous $MgSO_4$:NaCl (1 g:0.25 g) was added. After centrifugation, the organic extract was directly injected into the GC system.

The organic extracts were injected using the injection mode that provided the optimal chromatographic resolution and sensitivity with reproducible results was selected. The recoveries were calculated by comparing the signals provided by the extracts with the signals obtained on injecting solutions of the analytes prepared in each of the solvents with the same concentrations as those used in spiking the soils. Each solution was analysed in triplicate and the average value of the three injections was used.

The results obtained in these experiments are represented in Fig. 4. Several effects can contribute to these results. The hydration of $MgSO_4$ is an exothermic process, causing the sample extract to get hot during the extraction/partitioning step (temperatures between 40 and 45 °C). The low octanol–water partition coefficients (K_{ow}) for some of these compounds involve a possible high partition in the water phase and as a consequence low concentration in the analysed organic phase. Besides, the value of the organic

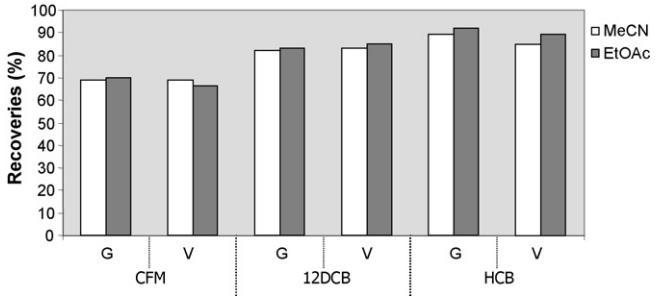

Fig. 4. Average recoveries of selected compounds from garden soil (G) and Vertisol (V).

carbon partition constant (K_{oc}) are very different for different compounds and could affect to the final recovery.

The high volatility of chloroform and its low value of K_{ow} could explain the low recoveries (between 66 and 70%) of this compound. For the semivolatile compounds the extraction recoveries are higher and should be mainly related to their polarity and interaction with soil. In this case, recoveries are between 83 and 92% and appear directly related to the polarity of the analytes. The strong binding to soil of hexachlorobenzene does not seem to be an important parameter as it presents the highest recovery. A similar behaviour with QuEChERS approach has been observed by other authors for compounds that present strong binding to soils [35].

Regarding the two different matrices, it can be observed that the extraction power of the technique in different complex soil samples is very similar. The recoveries found in the two soils showed no significant differences. This behaviour reinforces the idea that the binding of compounds to the soil is not a determining parameter in the extraction process given that the organic matter content in both soils is very different, but the recoveries obtained are similar.

Therefore, regarding the extraction from soil samples, the two studied solvents behave in a similar way. The better chromatographic behaviour observed for ethyl acetate led us to choose this solvent as the optimum.

In the application of the method to dry matrices, it is very common to add a volume of water to the samples, prior to the extraction step, to hydrate them and make the pores in the sample more accessible to the extraction solvent [13–15]. The effect of the moisture of the sample was studied by adding different volumes of water to the soil sample on the recoveries of the target compounds. Volumes of 1.5 and 2.5 mL of ultrapure water were added to the 2.5 g aliquots of the spiked garden soil sample, and the mixtures were vortex mixed for 1 min. Following this, the extraction procedure, using the amounts and proportions recommended in the original QuEChERS, was applied to the homogenized samples. The recoveries obtained were 65 and 67% for chloroform, 81 and 79% for 1,2-dichlorobenzene, and 91 and 93% for hexachlorobenzene, respectively. Comparison of these values by using a paired *t*-test showed that there were not significant differences in the recoveries of the compounds for the volumes of water studied. Therefore, we decided to choose a volume of 1.5 mL, which was enough to completely saturate the sample and appropriate to provide a proper homogenization of the sample during the vortex-mixing step.

Sample size is another of the commonly studied variables. Ideally, analytical methods try to reduce sample size to a minimum amount that provides statistically reliable results. Methods in which excessive sample size are used require larger solvent volumes, thus leading to more waste, greater safety concerns, greater storage, more labour and time, and more expense than necessary.

In this study two different sample sizes were selected: 2.5 and 5.0 g of soil. Water volume and salt proportions were scaled accordingly. The samples were extracted under the same conditions as previously with 2.5 mL of extracting solvent. Thus the sample:solvent ratios studied were 1:1 and 2:1. Larger sample sizes or larger solvent volumes were not possible because the glass centrifuge tube volume (15 mL) prevented proper homogenization of the sample and adequate extraction of the analytes during shaking process.

The results obtained show that signals obtained for a 2:1 sample:solvent ratio were 1.87–1.98 times higher compared to the signals for a 1:1 ratio. These results show that, if lower detection limits are needed and sample availability is not the limiting factor, different sample:solvent ratios could be designed to obtain concentration of the analytes ensuring at all times the right conditions for the extraction.

In the initial QuEChERS publication, after the initial single-phase extraction with MeCN, salts ($MgSO_4$ and NaCl) were added to induce phase separation. The salting-out effect resulting from addition of NaCl usually leads to increased recoveries of polar compound and allows to control the percentage of water in the organic phase. $MgSO_4$ was added at amounts well exceeding its saturation in water because of its ability to bind large amounts of water and thus significantly reduce the water phase and promote partitioning of the analytes into the organic phase.

Table 2
Influence of different combinations of salts on the recoveries of the target compounds (2.5 g soil sample).

Salts		Normalized peak area (RSD, %)[a]		
$MgSO_4$ (g)	NaCl (g)	CFM	1,2-DCB	HCB
1[b]	0	1.00 (0.5)	0.95 (0.57)	0.94 (0.38)
	0.25[b]	**1.00** (**0.5**)	**1.00** (**0.41**)	**1.00** (**1.08**)
	0.5	1.04 (4.9)	0.99 (0.63)	0.99 (0.25)
2	0	0.93 (1.4)	0.97 (0.57)	0.97 (1.02)
	0.25	0.99 (1.1)	0.97 (1.00)	0.98 (1.21)
	0.5	1.08 (1.0)	0.97 (0.41)	1.0 (0.83)

[a] $n = 3$.
[b] Combination of salts used in QuEChERS original.

In this work we have studied the effect of the addition of salts in the modified QuEChERS proposed. The first experiment was carried out without adding any salt. In this case, the upper layer is not transparent owing to the solubility of water in ethyl acetate. Therefore, different combinations of $MgSO_4$ with and without NaCl were studied in the extraction of RTC-CRM631 reference soil (with certified content for chloroform) and fortified garden soil samples (for 1,2-dichlorobenzene and hexachlorobenzene). Table 2 gives the results of the different experiments designed to determine this effect. Data are the average value of three determinations. Values in parentheses show the relative standard deviation for three replicates.

The peak areas obtained when the combination of salts recommended in the original QuEChERS method (1 g of $MgSO_4$ and 0.25 g of NaCl for 2.5 g of sample) is used have been assigned a value of 1.00 (in bold in Table 2), the values for the other assayed combinations being normalized to this value. It can be seen that there are no significant differences between the different combinations of salts studied. Moreover, there are no differences between the compound studied in the certified material (unspiked in the laboratory) and the analytes in the garden soil (polluted in the lab). The addition of NaCl does not have any significant effect in the soil matrices (the chromatograms are clean and no peaks from other compounds are observed, in contrast with the results reported for pesticides in food samples). Accordingly to these results and in order to simplify the new approach as much as possible only 1.0 g of $MgSO_4$ was used in the final procedure.

3.3. Analyte recoveries and reproducibility

We conducted recovery and reproducibility studies with the final method for the target analytes at different concentrations in two different matrices (garden soil and Vertisol). These concentrations are within the range of linearity of the method (20–600 μg/kg for CFM, 50–2400 μg/kg for 1,2-DCB, and 10–400 μg/kg for HCB) and well above the detection limits (2.2, 1.3, and 0.15 μg/kg, respectively). The calculated bias was below 5% for the three compounds studied. Table 3 shows the results obtained for the two fortified soil samples at different levels according to their sensitivity on the detector. The results correspond to the average of three injections on the chromatographic system in the optimal injection mode for each compound. Values in brackets indicate the RSD of these three measures. Recoveries were calculated by analyzing solutions of the compounds in EtOAc at the same concentration levels than those used to spike the soil samples; the values found were similar in the

Table 3
Percentage of recoveries and RSDs for the target compounds in garden soil and Vertisol.

Compound	Concentration level (μg/kg)	Garden soil		Vertisol	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
CFM	50	62	2.0	62	1.2
	200	68	1.8	64	0.5
1,2-DCB	625	82	1.0	83	3.4
	1562	78	1.0	79	1.5
HCB	15	93	1.9	86	1.6
	125	92	0.1	90	0.3

Table 4
Reproducibility of the global procedure proposed ($n = 10$).

Compound	Concentration level (μg/kg)	RSD (%)
CFM	50	7.6
1,2-DCB	19.5	3.5
HCB	0.46	3.3

two different matrices at the two fortified levels and satisfying the 62–93% recovery range with a relative standard deviation lower than 3.5% in the injection step. Again, the lowest recoveries corresponded to the most volatile compound (CFM). On the contrary the highest were obtained for the less volatile one (HCB).

In order to calculate the reproducibility of the overall approach 10 aliquots of a soil sample were submitted to the extraction procedure and the extracts were injected using the optimized method. The reproducibility, at concentrations specified in Table 4, was very good in all cases, with standard deviation values between 3.3 and 7.6%. Even so, the highest values correspond to the volatile compound, reflecting the difficulty in the extraction and determination of this kind of analytes.

4. Conclusions

A modified and simplified QuEChERS approach has been evaluated for the determination of chlorinated compounds in soil matrices.

Both MeCN and EtOAc can be used for analyte extraction, although EtOAc is preferred because it shows chromatographic advantages. Different injection techniques have been evaluated with good results in all cases.

The proposed method does not require a clean-up step and single liquid–liquid partitioning is achieved with the addition of just $MgSO_4$ to the sample:solvent mixture.

Future work must be developed to address more extensive validation of this method in order to extend it to different organic compounds in soil matrices.

Acknowledgments

The authors acknowledge the financial support of the DGI (CTQ2007-63157/BQU) and the Consejería de Educación y Cultura of the Junta de Castilla y León (Projects SA112A08 and Q3718001E) for this research. S.H.M. and A.M.C.F. are also grateful to Spanish MEC for award doctoral fellowships.

References

[1] N. Jakubowska, B. Zygmunt, Z. Polkowska, B. Zabiegała, J. Namieśnik, J. Chromatogr. A 1216 (2009) 422.
[2] B. Gilbert-López, J.F. García-Reyes, A. Molina-Díaz, Talanta 79 (2009) 109.
[3] T. Hyötyläinen, J. Chromatogr. A 1186 (2008) 39.
[4] W. Wardencki, J. Curyło, J. Namieśnik, J. Biochem. Biophys. Methods 70 (2007) 275.
[5] K. Demeestere, J. Dewulf, B. De Witte, H. Van Langenhove, J. Chromatogr. A 1153 (2007) 130.
[6] T. Hyötyläinen, M.-L. Riekkola, Anal. Chim. Acta 614 (2008) 27.
[7] T. Barri, J.A. Jönsson, J. Chromatogr. A 1186 (2008) 16.
[8] M. Anastassiades, S.J. Lehotay, D. Stajnbaher, F.J. Schenck, J. AOAC Int. 86 (2003) 412.
[9] S.J. Lehotay, A. De Kok, M. Hiemstra, P. Van Bodegraven, J. AOAC Int. 88 (2005) 595.
[10] S.J. Lehotay, K. Matovska, S.J. Yun, J. AOAC Int. 88 (2005) 630.
[11] K. Mastovská, S.J. Lehothay, J. Chromatogr. A 1040 (2004) 259.
[12] S.J. Lehotay, K. Mastovská, A.R. Lightfield, J. AOAC Int. 88 (2005) 615.
[13] C. Díez, W.A. Traag, P. Zommer, P. Marinero, J. Atienza, J. Chromatogr. A 1131 (2006) 11.
[14] T.D. Nguyen, E.M. Han, M.S. Seo, S.R. Kim, M.Y. Yun, D.M. Lee, G.H. Lee, Anal. Chim. Acta 619 (2008) 67.
[15] U. Koesukwiwat, K. Sanguankaew, N. Leepipatpiboon, Anal. Chim. Acta 626 (2008) 10.
[16] F.J. Schenck, A.N. Brown, L.V. Podhorniak, A. Parker, M. Reliford, J.W. Wong, J. AOAC Int. 91 (2008) 422.
[17] M.A. Aramendía, V. Borau, F. Lafont, A. Marinas, J.M. Marinas, J.M. Moreno, F.J. Urbano, Food Chem. 105 (2007) 855.
[18] A. Garrido Frenich, J.L. Martínez Vidal, E. Pastor-Montoro, R. Romero-González, Anal. Bioanal. Chem. 390 (2008) 947.
[19] K. Banerjee, D.P. Oulkar, S. Dasgupta, S.B. Patil, S.H. Patil, R. Savant, P.G. Adsule, J. Chromatogr. A 1173 (2007) 98.
[20] J.F. Garcia-Reyes, M.D. Hernando, C. Ferrer, A. Molina-Diaz, A.R. Fernandez-Alba, Anal. Chem. 79 (2007) 7308.
[21] P. Payá, M. Anastassiades, D. Mack, I. Sigalova, B. Tasdelen, J. Oliva, A. Barba, Anal. Bioanal. Chem. 389 (2007) 1697.
[22] D.A. Lambropoulou, T.A. Albanis, Anal. Bioanal. Chem. 389 (2007) 1663.
[23] A. Hercegova, M. Domotorova, E. Matisova, J. Chromatogr. A 1153 (2007) 54.
[24] C. Lesueur, P. Knittl, M. Gartner, A. Mentler, M. Fuerhacker, Food Control 19 (2008) 906.
[25] T. Cajka, J. Hajslova, O. Lacina, K. Mastovska, S.J. Lehotay, J. Chromatogr. A 1186 (2008) 281.
[26] T.D. Nguyen, J.E. Yu, D.M. Lee, G.H. Lee, Food Chem. 110 (2008) 207.
[27] S.J. Lehotay, J. AOAC Int. 90 (2007) 90.
[28] F. Plössl, M. Giera, F. Bracher, J. Chromatogr. A 1135 (2006) 19.
[29] C.K. Fagerquist, A.R. Lightfield, S.J. Lehotay, Anal. Chem. 77 (2005) 1473.
[30] K. Mastovska, A.R. Lightfield, J. Chromaotgr. A 1202 (2008) 118.
[31] A. Posyniak, J. Zmudzki, K. Mitrowska, J. Chromatogr. A 1087 (2005) 259.
[32] M.M. Aguilera-Luiz, J.L. Martínez Vidal, R. Romero-González, A. Garrido Frenich, J. Chromatogr. A 1205 (2008) 10.
[33] G. Stubbings, T. Bigwood, Anal. Chim. Acta 637 (2009) 68.
[34] B. Kinsella, S.J. Lehotay, K. Mastovska, A.R. Lightfield, A. Furey, M. Danaher, Anal. Chim. Acta 637 (2009) 196.
[35] C. Lesueur, M. Gartner, A. Mentler, M. Fuerhacker, Talanta 75 (2008) 284.