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Optimization of supercritical fluid extraction of organochlorine pesticides from real soil samples

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Abstract

The optimization of supercritical fluid extraction (SFE) of organochlorine pesticides from real soil samples is performed, according to a general stepwise set-up for quantitative SFE. Optimal conditions obtained from experiments with spiked samples were applied on real soil and the influence of several extraction parameters was tested to gain maximum concentrations of components in comparison with solvent extraction.

The optimal conditions achieved for spiked samples did not yield maximum concentrations for field samples. Stronger extraction conditions were necessary to overcome interactions between matrix and analytes. Longer dynamic extraction times were needed and the use of modifiers appeared to be essential for SFE of real samples. An increase in extraction pressure did not have any influence on extraction results. Comparable results were obtained for SFE and solvent extraction with an overall standard deviation between both methods of 25%.

The stepwise approach is useful in method development to visualize several aspects of SFE, such as the initial conditions, the importance of extraction parameters and the success of SFE for a specific compound–matrix combination. Possibilities in quantitative SFE are discussed, as well as restrictions in the break-through of SFE as technique for future sample pretreatment of solid samples.

1. Introduction

Several studies have shown the potential of supercritical fluid extraction (SFE) for the extraction of organic contaminants from soil. Originally, a lot of qualitative studies were reported, showing the effects of extraction at different densities on groups of components or reporting SFE of spiked samples only. Unfortunately, many studies seem to stop after the analysis of spiked samples [1–4]. More recent studies emphasize the importance of the differences between the investigations on spiked samples and field samples. In most cases, addition of modifiers or stronger extraction conditions seemed to be necessary to obtain SFE results which can be compared with conventional extraction techniques or with values of certified reference materials. Paschke et al. [5] optimized the SFE of polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs from diesel particulates with different kinds of fluids (CO_2 and $CHClF_2$) and modifiers (methanol and toluene), whereas Dankers et al. [6] improved PAH results in soil with the addition of relatively large volumes of dichloromethane. The quantitative extraction of polychlorinated biphenyls (PCBs) from river sediment was improved with supercritical

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$CHClF_2$ or with methanol-modified CO_2 [7], as was also reported by Onuska and Terry [8]. In most studies, the extraction parameters were optimized varying parameter by parameter, Van der Velde et al. [9] showed the application of an experimental design in the extraction of triazines from soil.

Hawthorne et al. [10] discussed a general set-up for quantitative SFE. Basic parameters in SFE, the partitioning of the analyte in the fluid, the sweeping from the extraction cell and the collection method, can be best determined by spiking on inert material excluding matrix influences. Only the solubility of the components in the supercritical fluid is tested, as well as the extraction time and the collection device and solvent. In our previous study we concluded that the choice of the collection solvent can be very critical, especially for the more volatile components [4]. Next, recoveries were determined of spiked samples and then SFE was performed on real samples in comparison with conventional extraction techniques.

In this study, a quantitative method for SFE of organochlorine pesticides (OCPs) in real soil samples is developed using this general set-up for quantitative SFE of organic components, based on sequential optimization of extraction parameters. Experiments were started from earlier investigations of our laboratory, in which optimal conditions for SFE of PCBs and OCPs in different types of soil were determined with spiked samples [4]. Several parameters were investigated for optimization of SFE, to equal or to improve the results from solvent extractions.

Finally, the possibilities and restrictions of SFE will be discussed as technique for future pretreatment of soil samples.

2. Experimental

2.1. Samples

Soils were air dried, allowed to pass through a 2.8-mm sieve and subsequently homogenized in a ball mill. Field samples were obtained from the Dutch monitoring programme on soil, concerning soils with about 5% organic carbon used for grass land, agriculture land and orchard soil, respectively. Blank soils were characterized as sand and peat soil, with 0.3 and 3.3% organic carbon, respectively. Spiked samples were prepared just before analysis, waiting 15 min till 1 h to allow evaporation of the solvents (the evaporation time depended on the amount of solvents used) with the following components: α-hexachlorocyclohexane (α-HCH), hexachlorobenzene (HCB), β-HCH, γ-HCH, β-heptachloroepoxide (β-HEPO), 2,2-bis(p-chlorophenyl)-1,1-dichloroethene (p,p'-DDE), dieldrin, 2,2-bis(p-chlorophenyl)-1,1-dichloroethane (TDE), o,p'-DDT, p,p'-DDT and several PCBs. PCBs were from CIL (Cambridge Isotope Labs., Woburn, MA, USA), OCPs from Promochem (Wesel, Germany). Spiking levels were chosen typically between 1 and 10 ng/g of dry soil.

2.2. Extraction procedures

Solvent extraction

Aliquots of 15 g of soil were extracted two times with 40 ml of acetone during 30 min using a shaking machine. The organic layer was separated by centrifugation. The liquid fractions were mixed with 800 ml of water and a few ml of saturated sodium chloride, and were then extracted twice with 50 ml of hexane. The combined hexane fractions were dried and, after addition of internal standards (PCBs 44 and 141), concentrated in a Kuderna–Danish apparatus till 10 ml. In some cases, a clean-up is performed to remove interferences from sulphur components. All solvents used were pesticide-grade (hexane) or distilled (acetone).

Supercritical fluid extraction

Supercritical fluid extractions were performed on a Carlo Erba (Milan, Italy) SFC 3000 instrument using a double 70-ml syringe pump (SFC 300) and an SFE-30 extraction unit. Extractions were performed using constant pressure and constant temperature. Various extraction times were programmed using combinations of static and dynamic extraction conditions. The specific extraction conditions are given in the tables. Supercritical pressure was maintained inside the extraction vessel using a deactivated fused-silica

2 m × 50 μm I.D. restrictor (SGE, Austin, TX, USA), resulting in a liquid CO_2 flow of 160–180 μl/min (at 20 MPa).

Accurately weighed soil samples were brought into a 0.5-ml extraction cell, filled up at one side with a thin layer of quartz sand to prevent clogging of the system and stamped to achieve homogeneous packing. Solvent collection was performed into a 2-ml vial containing approximately 1 ml of isooctane, with a known concentration of internal standard mixture (PCBs 44 and 141). Modifier was added directly to the extraction cell, just before supercritical extraction. CO_2 was SFC grade from Air Products & Chemicals (Waddinxveen, Netherlands).

2.3. Analysis

A Hewlett-Packard (HP) 5890 gas chromatograph, equipped with an HP 7673A autosampler, an electron-capture detector, and an Ultra-2 (50 m × 0.2 mm I.D.; 0.33 μm; HP) or DB-5 (30 m × 0.25 mm I.D.; 0.25 μm; J & W Scientific, Folsom, CA, USA) column was used for chromatographic separation, interfaced to an HP 3365 Chemstation (Hewlett-Packard, Palo Alto, CA, USA). Helium was used as carrier gas (2 ml/min) and argon–methane or nitrogen as purge gas (60 ml/min). After injection of 2–4 μl, the temperature programme consisted of an initial temperature of 80°C in several steps to the final temperature of 290°C. The injector temperature was 250°C and detector temperature was 325°C. Quantification was performed by comparison with an external standard mixture, using PCBs 44 and 141 as internal standards. Limits of determination for each component were 0.5 ng/g of soil, using the conditions specified above for sample preparation and analysis.

3. Results and discussion

3.1. Optimization of extraction parameters

In earlier investigations, we determined the optimal conditions for SFE of PCBs and OCPs. Analytes were first spiked on glass beads to test the initial extraction conditions. Restrictor, pressure, extraction time and collection solvent were adapted. Next, the analytes were spiked on different types of soil and good recoveries were found (Table 1), especially for soil with high contents of organic carbon usually giving low recoveries when solvent extraction is applied, because of binding of analytes to the soil matrix [4]. In the framework of this study, extraction times were varied from 10 min static and 20 min dynamic (SFE 10/20) to 30 min dynamic extraction (SFE 30) (Table 1). For sand no effect of extraction times was found, but for peat soil a decrease in recoveries of 10 to 20% for all components was found going to longer dynamic extraction times. With respect to the identical results for sand, this cannot be an effect of the collection method. A possible explanation is the difference in kinetics of the partitioning process for components to become available from sand or from peat soil.

Starting from the optimized conditions, a first series of extraction experiments with three different field soils was performed. In Table 2, the results of the solvent extraction and SFE are given for the organochlorine pesticides (no PCBs were found), using as extraction conditions: 20 MPa, 50°C, 10 min static and 20 min dynamic extraction times and pure CO_2. In a second series, the extraction time was changed to 30 min dynamic extraction to increase recoveries.

The SFE results of the field soils are in the same order of the concentrations found after solvent extraction. In this regard it must be realized, that concentrations are not corrected for extraction recoveries. For the measured components liquid–liquid extraction recoveries ranged from 50 to 80%, whereas for SFE recoveries were between 91 and 107% (Table 1). Going to longer dynamic extraction times in SFE, a slight improvement can be seen (a further increase in dynamic extraction time did not give any better extraction results). This is in contradiction with the SFE for spiked samples. Apparently, the binding of the components to the matrix and extraction process cannot be compared for real samples and spiked samples with regard to diffusion, partitioning and kinetics.

Table 1

Recovery experiments with liquid–liquid extraction (LLE) and SFE of PCBs and OCPs on sand and peat soil ($n = 3$)

Component	Addition (ng/g)	Sand						Peat soil					
		LLE		SFE 10/20		SFE 30		LLE		SFE 10/20		SFE 30	
		Recovery (%)	S.D. (%)	Recovery (%)	S.D. (%)	Recovery (%)	S.D. (%)	Recovery (%)	S.D. (%)	Recovery (%)	S.D. (%)	Recovery (%)	S.D. (%)
α-HCH	2.3	98.3	5.3	98.4	6.7	98.6	12.5	101.0	14.0	96.4	6.8	78.3	4.4
HCB	0.9	108.0	3.0	101.6	3.7	100.4	13.3	113.0	19.0	95.9	8.4	89.7	7.5
β-HCH	3.3	110.0	4.0	103.6	0.8	102.8	14.5	157.0	11.0	112.8	14.6	97.3	5.1
γ-HCH	2.2	118.0	8.0	103.1	4.7	103.7	12.2	109.0	12.0	91.2	7.7	81.8	5.5
β-HEPO	3.2	98.5	2.9	92.1	19.3	97.4	5.6	[a]	[a]	93.9	6.6	79.1	2.1
p,p′-DDE	5.1	86.7	3.8	99.2	1.4	94.2	8.1	75.6	12.6	107.2	3.8	97.6	11.0
Dieldrin	4.8	80.5	1.7	98.3	2.4	96.7	8.7	83.1	11.9	95.6	4.6	78.2	5.8
TDE	8.2	75.9	2.8	97.5	6.1	97.8	10.5	78.9	11.3	91.3	6.8	77.1	6.4
o,p′-DDT	8.9	84.5	3.9	97.1	3.4	95.9	8.7	50.7	10.5	98.3	6.4	82.5	7.7
p,p′-DDT	10.2	91.1	6.6	93.5	7.8	93.3	17.6	64.5	10.9	103.4	9.5	111.0	42.0
PCB 28	4.5	107.0	1.0	112.4	2.2	103.5	11.3	103.0	11.0	104.9	9.8	92.5	6.4
PCB 52	3.2	89.9	2.9	96.7	3.0	97.1	11.6	59.0	8.7	94.9	6.0	78.7	5.6
PCB 101	3.9	96.9	3.1	102.7	3.1	97.9	9.6	83.8	8.4	110.5	6.7	91.4	8.1
PCB 118	2.8	90.5	4.2	109.0	4.8	99.6	7.1	98.0	13.4	102.8	8.2	89.5	9.5
PCB 138	2.8	96.5	3.7	102.7	2.5	102.4	11.2	64.2	12.5	106.7	10.2	90.1	10.8
PCB 153	4.0	89.5	6.9	102.2	1.1	94.9	7.0	57.8	13.3	99.6	6.2	85.2	9.9
Average		95.1		100.6		98.5		81.2		100.3		87.5	

SFE conditions: 20 MPa; 50°C; CO_2; extraction time 10 min static and 20 min dynamic (SFE 10/20) or 30 min dynamic (SFE 30); flow ca. 160 ml/min; collection solvent isooctane.

[a] Interference.

Modifiers with different characteristics were added directly to the extraction cell for the orchard soil to try to improve the SFE results. In Table 3, concentrations are shown after addition of toluene, acetonitrile and methanol respectively. Toluene did not gave any improvement, but rather a decrease, whereas Mulcahey and Taylor [11] reported an increase for PCBs. Both acetonitrile and methanol give an increase in concentrations, from which methanol gives the best overall results. This is in agreement with Refs. [7] and [8]. Obviously, to overcome the interactions between the analyte and the matrix, introduction of modifier is essential. To test the influence of the amount of modifier, a series of 5 to 30 μl methanol was added to the extraction cell. In Table 4, the results are shown for the orchard soil. The amount of 20 μl methanol added to the extraction cell gave the highest concentration of OCPs. This amount corresponds with a volume percentage of about 10% with respect to CO_2 on basis of the free cell volume (total cell volume minus the sample volume). Probably, concentrations decrease by using higher amounts of modifier because the fluid is no longer supercritical above this value. Addition of modifier and using a combination of static and dynamic extraction times, does not give a better extraction yield, as can be seen in the last column of Table 3. This confirms the idea that the acting of the modifier is primarily the wetting of the matrix to facilitate the accessibility of the analytes and not an increase of diffusion.

Finally, the influence of pressure was tested. Table 5 shows that an increase in pressure did not had any influence on concentrations and therefore further extractions were performed at 20 MPa to diminish co-extraction of matrix components. Second extractions were performed

Table 2
Comparison of field samples with LLE and SFE with different extraction times (concentrations are not corrected for recoveries)

Component	Grass land						Agriculture land						Orchard soil					
	LLE ($n = 4$)		SFE 10/20 ($n = 3$)		SFE 30 ($n = 3$)		LLE ($n = 4$)		SFE 10/20 ($n = 3$)		SFE 30 ($n = 3$)		LLE ($n = 2$)		SFE 10/20 ($n = 2$)		SFE 30 ($n = 2$)	
	ng/g	S.D. (ng/g)	ng/g	S.D. (ng/g)	ng/g	S.D. (ng/g)	ng/g	S.D. (ng/g)	ng/g	S.D. (ng/g)	ng/g	S.D. (ng/g)	ng/g	S.D. (ng/g)	ng/g	S.D. (ng/g)	ng/g	S.D. (ng/g)
HCB	–	–	–	–	–	–	6.4	0.1	6.0	0.6	5.8	0.4	–	–	–	–	–	–
p,p'-DDE	4.1	0.3	4.4	0.4	5.5	1.1	1.1	0.0	1.6	0.2	1.5	0.2	63.1	–	50.3	–	56.2	–
TDE	6.1	0.5	0.8	0.2	1.7	0.4	–	–	–	–	–	–	12.2	–	12.8	–	20.1	–
o,p'-DDT	2.3	0.2	3.0	0.5	4.0	1.0	0.6	0.1	1.1	0.2	1.0	0.2	44.8	–	37.7	–	43.7	–
p,p'-DDT	11.4	1.7	21.9	2.4	29.7	4.6	–	–	5.5	0.4	6.1	0.3	260.6	–	236.8	–	296.4	–

SFE conditions: 20 MPa; 50°C; CO_2; extraction time: 10 min static and 20 min dynamic (SFE 10/20) or 30 min dynamic (SFE 30); flow ca. 160 μl/min. – = Not detectable (0.5 ng/g).

Table 3
SFE with addition of different modifiers on orchard soil

Component	SFE 30						SFE 10/20	
	Modifier toluene ($n = 3$)		Modifier acetonitrile ($n = 3$)		Modifier methanol ($n = 6$)		Modifier methanol ($n = 3$)	
	ng/g	S.D. (ng/g)	ng/g	S.D. (ng/g)	ng/g	S.D. (ng/g)	ng/g	S.D. (ng/g)
p,p'-DDE	48.2	2.1	68.4	5.7	72.0	2.9	59.5	0.6
TDE	24.5	0.7	32.9	1.6	20.6	0.8	16.9	0.3
o,p'-DDT	41.3	2.4	57.2	3.9	61.8	1.5	52.5	0.9
p,p'-DDT	224	11	325	19	355	8	353	48

SFE conditions: 20 MPa; 50°C; CO_2; 20 μl modifier; flow ca. 160 μl/min.

Table 4
Influence of the amount of methanol modifier on orchard soil

Component	5 μl MeOH, 2.4%[a] (n = 3)		10 μl MeOH, 4.8%[a] (n = 3)		15 μl MeOH, 7.1%[a] (n = 3)		20 μl MeOH, 9.5%[a] (n = 6)		25 μl MeOH, 11.9%[a] (n = 3)		30 μl MeOH, 14.3%[a] (n = 3)	
	ng/g	S.D. (ng/g)	ng/g	S.D. (ng/g)	ng/g	S.D. (ng/g)	ng/g	S.D. (ng/g)	ng/g	S.D. (ng/g)	ng/g	S.D. (ng/g)
p,p'-DDE	53.9	1.1	55.9	2.3	63.3	4.7	72.0	2.9	61.3	5.2	65.3	1.0
TDE	17.1	0.5	17.1	0.4	16.6	0.5	20.6	0.8	16.3	0.6	17.3	0.2
o,p'-DDT	42.9	1.0	44.5	1.9	52.3	3.6	61.8	1.5	51.8	5.0	56.6	0.8
p,p'-DDT	250	3	261	9	311	22	355	8	315	23	344	1

SFE conditions: 20 MPa; 50°C; CO_2; 30 min dynamic; flow ca. 160-180 μl/min.

[a] % (v/v) of the modifier with respect to CO_2, on basis of free cell volume.

Table 5
Influence of extraction pressure

Component	20 MPa, density 0.791, 160–180 μl/min (n = 6)		25 MPa, density 0.841, 190–210 μl/min (n = 2)		30 MPa, density 0.880 g/ml, 230–250 μl/min (n = 2)	
	ng/g	S.D. (ng/g)	ng/g	S.D. (ng/g)	ng/g	S.D. (ng/g)
p,p'-DDE	72.0	2.9	59.3	–	70.4	–
TDE	20.6	0.8	20.5	–	24.5	–
o,p'-DDT	61.8	1.5	49.7	–	57.6	–
p,p'-DDT	355	8	329	–	383	–

SFE conditions: 50°C; CO_2; 30 min dynamic; addition of 20 μl MeOH; orchard soil.

for several experiments described above, under the same extraction conditions, and no detectable amounts of components were found.

The orchard soil was extracted six times using the final SFE conditions (20 MPa, 50°C, 30 min dynamic extractions and 20 μl methanol as modifier), in different series of experiments over a longer time period, giving good reproducibilities varying from 20.6 ± 0.8 ng/g for TDE to 355 ± 8 ng/g for *p,p'*-DDT. A typical chromatogram of SFE of OCPs in orchard soil is shown in Fig. 1.

Some random soil samples were analysed using SFE with final conditions and solvent extraction. In Fig. 2, the results of both methods are plotted. Assuming a concentration-independent relative standard deviation between both methods, a pooled standard deviation of 25% was calculated for all soils, components and concentrations. So, comparable results were obtained for SFE and solvent extraction. More soils have to be analysed to make a final comparison between liquid–liquid extraction and SFE. The reproducibility of both methods is comparable (Table 2), the time needed for sample preparation is not faster using a non-automated SFE instrument, but the use of toxic and environmentally hazardous solvents is highly reduced.

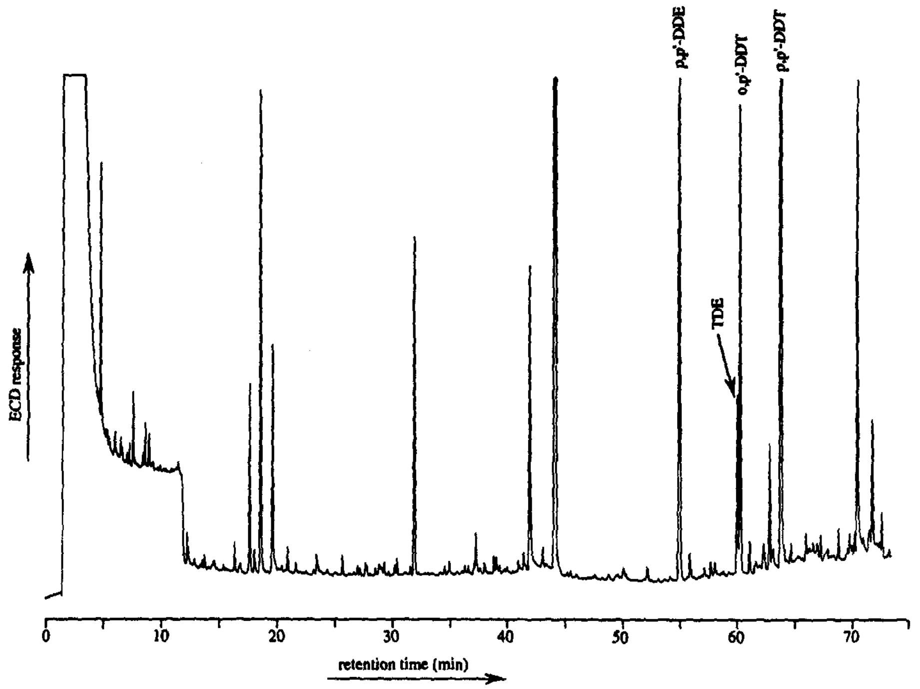

Fig. 1. GC–electron-capture detection (ECD) chromatogram of organochlorine pesticides in orchard soil using final SFE conditions. For chromatographic conditions see Experimental.

3.2. Possibilities and restrictions in quantitative SFE

The set-up of this study, using a stepwise approach for the optimization, offers several advantages. Information has been obtained about the relative importance of extraction parameters for these specific compounds, such as the solubility of the compounds by the chosen density, the transport in the SFE system and the collection method. By the next step, the spiking on the soil, the extraction of the compounds from the matrix can be studied. If no satisfactory results have been obtained in these steps, it is in principle useless to continue with SFE for this compound–matrix combination. On the other hand, it is important to avoid endless optimizations, because this process has to be repeated with the extraction of real soil samples, as described above. The SFE of real samples shows that stronger extraction conditions are necessary, because compound–matrix interactions are different for real samples, probably resulting from bound residues.

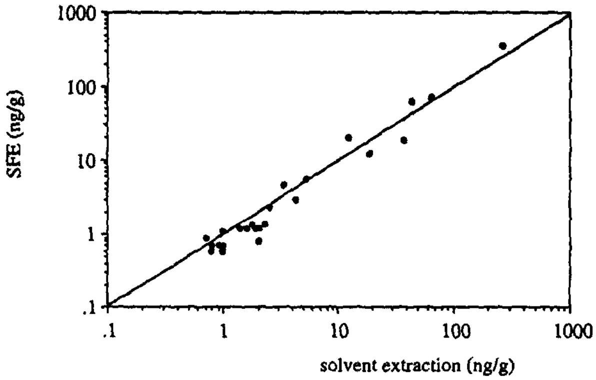

Fig. 2. Correlation of SFE with solvent extraction for several field samples and components.

A sequential as well as a statistical approach can be chosen for the optimization of extraction parameters for either spiked or real samples. The statistical approach is only appropriate, if it is known that SFE will be a suitable method for compounds and matrix. A statistical method offers the opportunity to study the effects of the different parameters with fewer experiments and to distinguish the interactions between parameters [9]. In practice, the sequential approach is applied more often, especially when chemometric support is not available, and offers the opportunity to adjust parameters during the experiments, as is shown in this study. So, the choice of the approach has to be dependent on the specific SFE problem.

In overlooking our results and other publications in the field of SFE, a major breakthrough of SFE has not been reached yet, while comparable results with conventional methods have been reported. In our opinion, SFE suffers from the same problems as, for example, solvent and Soxhlet extraction. It is difficult to achieve complete extraction from solid matrices like soil, because certain fractions of compounds will irreversibly bind to the matrix. Besides, in the case of soils, every soil sample is different in composition, requiring specific optimizations and determinations of recovery. However, in conventional techniques these aspects have been accepted and endless optimizations for complete extraction yields have been dropped. In using a new technique like SFE, a solution for these problems and also higher extraction efficiencies have been expected. At this moment, SFE is not ready to offer this solution, rather than an approximation of the results of conventional techniques.

4. Conclusions

A method development for SFE of OCPs in field samples has been performed using a general stepwise approach for quantitative SFE, starting with spiking on glass beads to establish initial conditions, followed by spiking on different kinds of soil to further adapt SFE parameters. As was expected, the conditions were not directly suitable for real soil samples. The component–matrix interactions in real samples behave different with respect to spiked samples as can be seen in adaptations in extraction times and the effects of modifiers. Finally, comparable results were obtained for SFE and solvent extraction. The stepwise approach gives a deeper understanding in the parameters which are important in development of quantitative SFE. As technique, SFE is not ready yet to overcome the general problems in the extraction of soil samples.

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