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Effects of parameters on supercritical fluid extraction of triazines from soil by use of multiple linear regression

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Abstract

The effects of various parameters on supercritical fluid extraction (SFE) of triazines from soil were studied with an experimental design, based on multiple linear regression. The SFE was performed using a multi-extraction unit with simultaneous extraction of eight samples. Different types of soil samples were spiked with a series of five triazines with different polarities.

The developed design was a compromise of various objectives like the relative importance of the different parameters, the total amount of experiments and instrumental limitations. Eleven series of experiments using different conditions were performed, resulting in a data set of over 200 data. Regression analysis was applied to evaluate the data set of each individual triazine component. Furthermore, the influence of the different parameters was tested, resulting in a limitation of the original parameter set as well as a combination of some parameters to avoid interactions. The influence of the pressure on the recovery appeared to be very important, recoveries increased with increasing pressures. The influence of the modifier was also essential, only when it was added directly to the extraction cell, and the effect is increasing with component polarity. The effects of the temperature and extraction time were slightly negative and not significant, whereas a small effect of the type of soil was observed. Two other models, combining the whole data set for all triazines, were applied resulting in a more pronounced effect of the individual parameters.

Multiple linear regression appeared to be a useful tool to study the effects of the many parameters in SFE, in order to reduce the number of experiments, to facilitate the evaluation of data and to distinguish possible interactions between several parameters.

1. Introduction

In the past few years, supercritical fluid extraction (SFE) has received widespread attention in the field of analysis of organic contaminants in environmental samples. After many qualitative applications, SFE is now ready to prove its potential advantages as a quantitative and fast alternative for conventional extraction techniques such as liquid–liquid and Soxhlet extraction. Recent reviews have shown its applicability for a wide range of analytes in all kinds of matrices [1–5].

The advantages of SFE are the high efficiency and selectivity, the short extraction times, the simple concentration steps and the reduction in toxic and environmentally hazardous solvents. However, some advantages of SFE still have to be proved, like the ability to reduce the cost per

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analysis and on-line coupling with chromatographic techniques in routine analysis.

A challenge in SFE is the choice in and optimization of extraction parameters. Important parameters are pressure, temperature, amount and type of modifier, extraction time and cell volume. The extraction efficiency is directly depending on the solubility of the analytes in the fluid, which is determined by the density (pressure and temperature) and the vapour pressure of the analytes (temperature), and on the modifier. The modifier can be premixed with the fluid or added directly to the extraction cell. In the first case the modifier will increase the solubility of the analytes in the fluid, whereas in the second case the displacement of the analytes from the matrix active sites is facilitated [2]. It is suggested that a better wetting of the sample will enhance the accessibility of the analytes. The variability of the pressure and temperature will be determined by the supercritical point of the fluid, with or without addition of modifier, and by instrumental limitations. The extraction time determines, in combination with the restrictor dimensions and the cell volume, the total amount of supercritical fluid passing the cell.

In many publications, these extraction conditions are established empirically, due to a lack of solubility data of analytes in the supercritical fluids, especially when modifiers are added. Because of the complexity of the extraction process and the multitude of parameters, a solution is to use a statistical approach to reduce the number of experiments, to facilitate the interpretation of the data and to distinguish the possible interactions between several parameters. In most cases, a relatively simple approach is chosen starting with the two or three most important parameters in two settings, resulting in a 2^2 or 2^3 factorial design [6,7]. Ho and Tang [7] describe a 2^3 factorial design for three parameters (pressure, temperature and extraction time) at two levels (low and high) for the determination of polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides from cartridges. The result was used as the initial condition for a variable-size simplex optimization. Because only three parameters were varied, at the end it was necessary to add a modifier to improve recoveries for the heavier PAHs. Lopez-Avila et al. [8] applied a model with seven variables (pressure, temperature, moisture, cell volume, sample size, extraction time, modifier volume) at two levels (low and high) to determine group differences between the low and high values. Finally, 1/16th of the full design (8 experiments) is performed, so only a ranking of main effects and no statistical significance were reported.

In this study, a statistical approach of parameter settings using multiple linear regression is presented, primarily to show the effects of extraction parameters in SFE of triazines from soil. Secondly, the SFE instrument was only available during a short time period and therefore all experiments had to be planned beforehand, because GC analyses were performed afterwards. An experimental design offers the opportunity to make a plan for experiments, based on expert knowledge on the importance of the parameters.

1.1. Experimental design

For the design of the experiments the following arguments had to be considered.

(1) The main objective of the study was to establish the effects of a number of parameters on the SFE of triazines from soil. These are both the SFE parameters mentioned above and the types of soil.

(2) The relative importance of the parameters may differ considerably. Some are of crucial importance, as stated above, others are only incorporated because their influence could not be excluded beforehand.

(3) Series contain seven or eight experiments with equal instrumental settings.

(4) The reproducibility was expected to be large ($>10\%$), so interpretation of individual experiments would be difficult.

The difference in importance of the parameters is in contradiction with the most commonly used experimental designs which are symmetrical in the parameters. Therefore, instead of the conventional symmetrical designs another

Table 1
Parameter settings in experimental design

Parameter	Degrees of freedom	Settings
Pressure (MPa)	1	20, 25, 30, 50
Temperature (°C)	1	50, 70, 80, 90, 100
Extraction time (min)	1	30, 35, 40, 60, 70
Type of modifier	2[a]	MeOH, mixed CO_2–MeOH, mixed CO_2–acetone
Amount of modifier (μl)	1	100, 200, 300, 1000
Cell volume (ml)	1	3.5, 10
Amount of triazines (μl)	1	(25), 50, 100, (200), (250), (300), (400)[b]
Type of soil	2	Sand, peat, clay

[a]Only addition of MeOH used in model evaluation.
[b]Amounts of modifier used for linearity experiment only are given in parentheses.

strategy was chosen. On the basis of experimental knowledge and literature on SFE, a first concept of the design was made. The design was modified to optimize the statistical properties using multiple linear regression and simulated and measured data for SFE of triazines.

Finally, an experimental design was chosen based on ten parameters (and a constant), which is a compromise between the various objectives of this study. In Table 1 an overview is given of these parameters and the settings, whereby the more important parameters are included in all experiments.

2. Experimental

2.1. Samples

Three types of blank soil with different contents of organic carbon were used, namely sand, peat and clay, with 0.3, 3.3 and 6.8% organic carbon, respectively. In addition, field samples were collected at different time intervals after treatment with atrazine from two locations, Bergeijk (code B1A/B and B2A/B) and Laren (code L1A/B and L2A/B), both in the Netherlands.

All soils were dried at 40°C, passed through a 2.8-mm sieve and were subsequently homogenized in a ball mill. The blank soils were used for spiking experiments with triazines, i.e. simazine, atrazine, terbuthylazine and the metabolites desisopropylatrazine and desethylatrazine at concentrations of 30–170 μg/kg of soil for each component. Individual soil samples were spiked by adding a standard solution of triazines to the extraction cell, containing a subsample of the soil.

2.2. Standard materials

Highly pure standard materials (purity >98%) of simazine, atrazine, terbuthylazine, desisopropylatrazine and desethylatrazine were obtained from C.N. Schmidt (Amsterdam, Netherlands). Methanol was HPLC grade (J.T. Baker, Deventer, Netherlands) and ethyl acetate and acetone were nanograde (Promochem, Wesel, Germany).

CO_2 was SFE/SFC grade (Air Products & Chemicals, Waddinxveen, Netherlands).

2.3. Supercritical fluid extraction

Supercritical fluid extractions were performed on a Dionex (Salt Lake City, UT, USA) SFE-703 multi-extraction instrument, suitable for the simultaneous extraction of eight samples, with a SFE-703M co-solvent addition module, made available by Dionex Breda (Breda, Netherlands). The specific experimental conditions as extraction temperature, pressure, cell volume, extraction times etc., for the different experi-

ments are given in the tables. Some parameters were not varied due to instrumental or other limitations, e.g. the type of supercritical fluid, restrictor dimensions, cell geometry and the collection device. Furthermore, the instrument only allowed dynamic and no static extractions.

Fused-silica restrictors in stainless-steel tubing with a controlled flow of 250 ml/min (gaseous CO_2) were used at temperatures of 180°C. The samples were collected in 10-ml vials with 5 ml ethyl acetate with a known concentration of internal standards [desmethrin and polychlorinated biphenyl (PCB) 171]. The vials, equipped with an inner tube, were cooled to°5C.

Exactly weighed soil samples (of about 6 g for 3.5-ml cells and 14 g for 10-ml cells) were put into the extraction cell, at one side filled with a thin layer of quartz sand to prevent clogging of the system. Cells were completely filled and stamped to achieve homogeneous packing, which is important to prevent dead volumes and channelling. In the spiking experiments, a small volume of triazines in acetone was added dispersed over the soil in the extraction cell. Next, the cell was purged with a nitrogen flow during 20 min to allow the solvent to evaporate, this preventing the spiking solvent to act as modifier. Modifiers were added either directly to the extraction cell, or premixed with CO_2 by the co-solvent pump. Finally, SFE extracts were thoroughly mixed and were subjected to GC analysis.

2.4. Analysis

A Carlo Erba HRGC 5300 gas chromatograph with nitrogen–phosphorus detection (NPD) system and splitless injector, equipped with a A200S autosampler (all Carlo Erba, Milan, Italy) and using a DB5 column (30 m × 0.32 mm I.D.; 0.25 μm; J & W Scientific, Folsom, CA, USA), was used for the quantitative analysis of the triazines. After injection of 3 μl and a splitless time of 45 s, the temperature programme consisted of an initial temperature of 80°C, 2 min hold, programmed to 170°C at 25°C/min, 8 min hold, then at 2°C/min to 190°C and at 15°C/min to the final temperature of 270°C and held for 15 min. The injector temperature was 220°C and detector temperature was 300°C. A GC–electron-capture detection (ECD) system consisting of an HP 5890 gas chromatograph, equipped with an HP 7673a autosampler and interfaced to an HP 3365 Chemstation (Hewlett-Packard, Palo Alto, CA, USA) using an Ultra 1 column (50 m × 0.25 mm I.D.; film thickness $d_f = 0.5$ μm; Hewlett-Packard) for confirmation. Quantification was performed by comparison with an external standard mixture, using desmethrin (GC–NPD) and PCB 171 (GC–ECD) as internal standards. Lower limits of determination for each component were 4 μg/kg, using the conditions specified above for sample preparation and analysis.

2.5. Statistical method

A linear model is used to describe the influence of the parameters on the recovery of the triazines. The model contains a constant (p_{11}) and the influence of 10 parameters ($p_1 \ldots p_{10}$):

$\boldsymbol{p} = p_1, p_2, \ldots p_{11}$ (p_{11} is the constant).

Then every experiment has 10 parameter settings described as:

$\boldsymbol{x} = x_1, x_2, \ldots x_{11}$ ($x_{11} = 1$).

The linear model, $M(\boldsymbol{x}, \boldsymbol{p})$, then reads:

$$M(\boldsymbol{x},\boldsymbol{p}) = p_1x_1 + p_2x_2 + \ldots p_{10}x_{10} + p_{11}$$

Some of the parameters in this model, like the various types of soil, have only two possible settings which are 1 for the soil type corresponding with the sample and 0 for the other soils. The settings of other parameters like pressure, temperature and extraction time have a continuum of possible settings. The linear model used does not incorporate terms of higher order like x_1^2 and interactions like x_1x_2. The incorporation of all these terms is not possible because then the number of terms would exceed the number of experiments.

In designing the experiments, the accuracy of the parameters of interest was calculated using simulated data, with the linear model both for the complete data set as for two subsets, which

contained only the first 35% and 70% of the data points (in case some experiments might be excluded for time reasons). Then, mutations in the designed parameter settings were applied to improve the accuracy of the important parameters both for the total data set as for the subsets. The information from the subsets was used to change the order in which the experiments were planned.

The linear model is calculated separately for each of the components analysed. The estimates of the parameters (p) and their standard deviation are calculated using (unweighed) multiple linear regression [9].

3. Results and discussion

The final experimental set up is consisting of eleven series of seven or eight simultaneous extractions, based on ten parameters with different settings (Table 1). The temperature is varied from 50 to 100°C, while the maximum pressure ranged from 20 to 50 MPa, because of instrumental limitations. The extraction time was chosen between 30 min for the 3.5-ml cell (only seven cells were available) and 70 min for the 10-ml cell. In all experiments different amounts of modifier were tested, methanol was added directly to the cell or CO_2 was premixed with methanol or acetone. From previous experiments on another SFE instrument (Carlo Erba SFC 3000), the initial conditions were established. From several collection solvents, the trapping efficiency of ethyl acetate proved to be the best. Further, it was shown that especially the use of a modifier is very important to increase the solubility of the triazines in CO_2. The best results were obtained with methanol. These results are in agreement with other SFE data on triazines in soil [10,11] and were used as initial conditions in this study. In two series the repeatability and linearity of the instrument and the supercritical fluid extraction were tested. The influence of the parameters was tested on different types of soil (sand, clay and peat) spiked with triazines and on field samples.

In Table 2, an overview is given of all series of experiments with the specific extraction conditions for pressure, temperature, extraction time, cell volume, amount and type of modifier, spike volume and the type of soil. The results are given as a percentage of recovery for recovery experiments and for field samples a concentration is calculated. Results below determination limits (LOD; 4 μg/kg for 10-ml cells and 9 μg/kg for 3.5-ml cells) are marked with an $<$. In the model calculation, these results have been given the value zero. A number of experiments was not successful, due to plugging and imperfections in the collection device, and is marked with an a. These experimental data were not incorporated in the linear model. In Fig. 1a, a typical GC–NPD chromatogram of triazines spiked on peat soil is given. This chromatogram shows that no matrix disturbances are present using SFE as extraction technique.

3.1. Repeatability and linearity

The study has been designed for evaluation with a model incorporating all relevant parameters, instead of comparisons between pairs of experiments to study single parameters. Two exceptions on this general idea were made deliberately: the repeatability and the linearity.

Therefore, two series of experiments were incorporated to study these items explicitly. In series 3 (Table 2), seven identical experiments were performed and in series 5, nearly identical experiments were performed with variation in the amount of added triazine standards. Calibration curves for all triazines have correlation coefficients of 0.981 and better.

In Table 3, the averages and standard deviation of the recoveries are shown for series 3 and 5. The average recovery of series 3 ranged from 9 to 66% with standard deviations of 7 to 21%, whereas series 5 ranged from 92 to 99% with standard deviations of 5 to 11% for the different triazines. Recoveries for series 5 were good, with moderate standard deviations for intra-series variations. Apparently, extraction conditions of series 5 were more close to the optimum. In addition, results may be influenced by the type of soil, clay (series 3) versus sand (series 5). It is

Table 2
Results of SFE of triazines in experimental design (recoveries (%) and concentrations (μg/kg) of triazines by selected conditions)

Series/ experiment No.	Soil type	Spike (μl)	Cell volume (ml)	Modifier	Pressure (MPa)	Temperature (°C)	Extraction time (min)	Triazines in recoveries (%)/concentrations (μg/kg)				
								Desisopropyl-atrazine	Desethyl-atrazine	Simazine	Atrazine	Terbuthyl-azine
1.1	Clay	100	3.5	100 μl MeOH	20	50	40	<	8%	17%	60%	55%
1.2	Clay	100		200 μl MeOH				<	27%	80%	79%	59%
1.3	Clay	100		300 μl MeOH				27%	54%	78%	52%	39%
1.4	Sand	–		100 μl MeOH				[a]	[a]	[a]	[a]	[a]
1.5	Sand	100		100 μl MeOH				<	<	<	<	<
1.6	Peat	100		100 μl MeOH				<	<	8%	10%	9%
1.7	Field B1B	–		100 μl MeOH				[a]	[a]	[a]	[a]	[a]
2.1	Clay	–	10	300 μl MeOH	20	70	60	[a]	[a]	[a]	[a]	[a]
2.2	Clay	50		300 μl MeOH				<	25%	31%	41%	39%
2.3	Clay	50		1 ml MeOH				34%	31%	44%	41%	40%
2.4	Sand	50		300 μl MeOH				34%	25%	101%	100%	96%
2.5	Sand	50		1 ml MeOH				82%	58%	73%	74%	64%
2.6	Field B1B	–		300 μl MeOH				<	<	<	26 μg/kg	<
2.7	Field B1B	–		1 ml MeOH				[a]	[a]	[a]	[a]	[a]
2.8	Sand	50		300 μl MeOH				[a]	[a]	[a]	[a]	[a]
3.1	Clay	50	3.5	100 μl MeOH	25	50	30	11%	26%	45%	74%	76%
3.2	Clay	50		100 μl MeOH				5%	37%	59%	58%	60%
3.3	Clay	50		100 μl MeOH				<	<	36%	55%	56%
3.4	Clay	50		100 μl MeOH				<	47%	68%	73%	72%
3.5	Clay	50		100 μl MeOH				<	12%	17%	64%	62%
3.6	Clay	50		100 μl MeOH				6%	39%	43%	68%	65%
3.7	Clay	50		100 μl MeOH				43%	60%	81%	73%	70%
4.1	Clay	–	10	300 μl MeOH	50	50	70	<	<	<	<	<
4.2	Clay	50		300 μl MeOH				<	58%	56%	56%	52%
4.3	Sand	–		300 μl MeOH				<	<	<	<	<
4.4	Sand	50		300 μl MeOH				107%	111%	113%	105%	105%

4.5	Peat	–		300 μl MeOH				<	<	<	<	<
4.6	Peat	50		300 μl MeOH				40%	115%	120%	108%	98%
4.7	Field B1B	–		300 μl MeOH				<	<	<	29 μg/kg	<
4.8	Field B1B	50		300 μl MeOH				91%	92%	84%	62%	80%
5.1	Sand	25	3.5	300 μl MeOH	30	70	35	90%	[a]	99%	97%	95%
5.2	Sand	50		300 μl MeOH				[a]	[a]	[a]	[a]	[a]
5.3	Sand	100		300 μl MeOH				93%	84%	96%	91%	89%
5.4	Sand	200		300 μl MeOH				109%	102%	103%	99%	95%
5.5	Sand	250		300 μl MeOH				87%	87%	102%	100%	96%
5.6	Sand	300		300 μl MeOH				92%	86%	89%	84%	84%
5.7	Sand	400		300 μl MeOH				113%	102%	103%	98%	93%
6.1	Clay	–	10	CO_2–MeOH (90:10)	30	80	70	<	<	<	<	<
6.2	Clay	50		CO_2–MeOH (90:10)				<	<	<	<	<
6.3	Sand	–		CO_2–MeOH (90:10)				<	<	<	<	<
6.4	Sand	50		CO_2–MeOH (90:10)				<	<	16%	57%	69%
6.5	Peat	–		CO_2–MeOH (90:10)				<	<	<	<	<
6.6	Peat	50		CO_2–MeOH (90:10)				<	<	31%	49%	53%
6.7	Field B1B	–		CO_2–MeOH (90:10)				<	<	<	9 μg/kg	<
6.8	Quartz	–		CO_2–MeOH (90:10)				<	<	<	<	<
7.1	Clay	–	3.5	CO_2–MeOH (95:5)	25	90	30	<	<	<	<	<
7.2	Clay	50		CO_2–MeOH (95:5)				<	<	<	<	<
7.3	Sand	–		CO_2–MeOH (95:5)				<	<	<	<	<
7.4	Sand	50		CO_2–MeOH (95:5)				<	<	7%	47%	73%
7.5	Peat	–		CO_2–MeOH (95:5)				<	<	<	<	<
7.6	Peat	50		CO_2–MeOH (95:5)				<	17%	58%	89%	94%
7.7	Field B1B	–		CO_2–MeOH (95:5)				<	<	<	12 μg/kg	<
8.1	Field B1B	–	10	300 μl MeOH	20	50	60	<	<	<	50 μg/kg	<
8.2	Field L1B	–		300 μl MeOH				<	<	<	41 μg/kg	<
8.3	Field L1A	–		300 μl MeOH				<	<	<	59 μg/kg	<
8.4	Field L2A	–		300 μl MeOH				<	<	<	48 μg/kg	<
8.5	Field L2B	–		300 μl MeOH				<	<	<	41 μg/kg	<
8.6	Field B1A	–		300 μl MeOH				<	<	<	30 μg/kg	<
8.7	Field B2B	–		300 μl MeOH				<	<	<	37 μg/kg	<
8.8	Field B2A	–		300 μl MeOH				<	<	<	22 μg/kg	<

(Continued on p. 132)

Table 2 *(continued)*

Series/ experiment No.	Soil type	Spike (μl)	Cell volume (ml)	Modifier	Pressure (MPa)	Temperature (°C)	Extraction time (min)	Triazines in recoveries (%)/concentrations (μg/kg)				
								Desisopropyl-atrazine	Desethyl-atrazine	Simazine	Atrazine	Terbuthyl-azine
9.1	Clay	–	3.5	100 μl MeOH	30	100	30	<	<	<	<	<
9.2	Clay	50		100 μl MeOH				<	<	22%	48%	56%
9.3	Sand	–		100 μl MeOH				<	<	<	<	<
9.4	Sand	50		100 μl MeOH				<	<	<	43%	57%
9.5	Peat	–		100 μl MeOH				<	<	<	<	<
9.6	Peat	50		100 μl MeOH				12%	32%	92%	111%	104%
9.7	Field B1B	–		100 μl MeOH				<	<	<	28 μg/kg	<
10.1	Clay	–	10	CO_2–acetone (90:10)	30	100	70	<	<	<	<	<
10.2	Clay	50		CO_2–acetone (90:10)				<	<	<	<	<
10.3	Clay	50		CO_2–acetone (90:10)				<	<	<	25%	29%
10.4	Sand	–		CO_2–acetone (90:10)				<	<	<	<	<
10.5	Sand	50		CO_2–acetone (90:10)				<	<	23%	46%	55%
10.6	Peat	–		CO_2–acetone (90:10)				<	<	<	<	<
10.7	Peat	50		CO_2–acetone (90:10)				<	<	37%	57%	67%
10.8	Field B1B	–		CO_2–acetone (90:10)				<	<	<	17 μg/kg	<
11.1	Clay	–	3.5	CO_2–acetone (90:10)	20	70	30	<	<	<	<	<
11.2	Clay	50		CO_2–acetone (90:10)				<	<	<	<	<
11.3	Sand	–		CO_2–acetone (90:10)				<	<	<	<	<
11.4	Sand	50		CO_2–acetone (90:10)				<	<	<	55%	82%
11.5	Peat	50		CO_2–acetone (90:10)				<	<	14%	68%	95%
11.6	Field B1B	–		CO_2–acetone (90:10)				<	<	<	<	<
11.7	Field L1B	–		CO_2–acetone (90:10)				<	<	<	<	<

Spike concentration: ca. 10 μg triazine component/ml. < = LOD: 4 μg/kg for 3.5-ml cell and 9 μg/kg for 10-ml cell.

[a] Experiment failed.

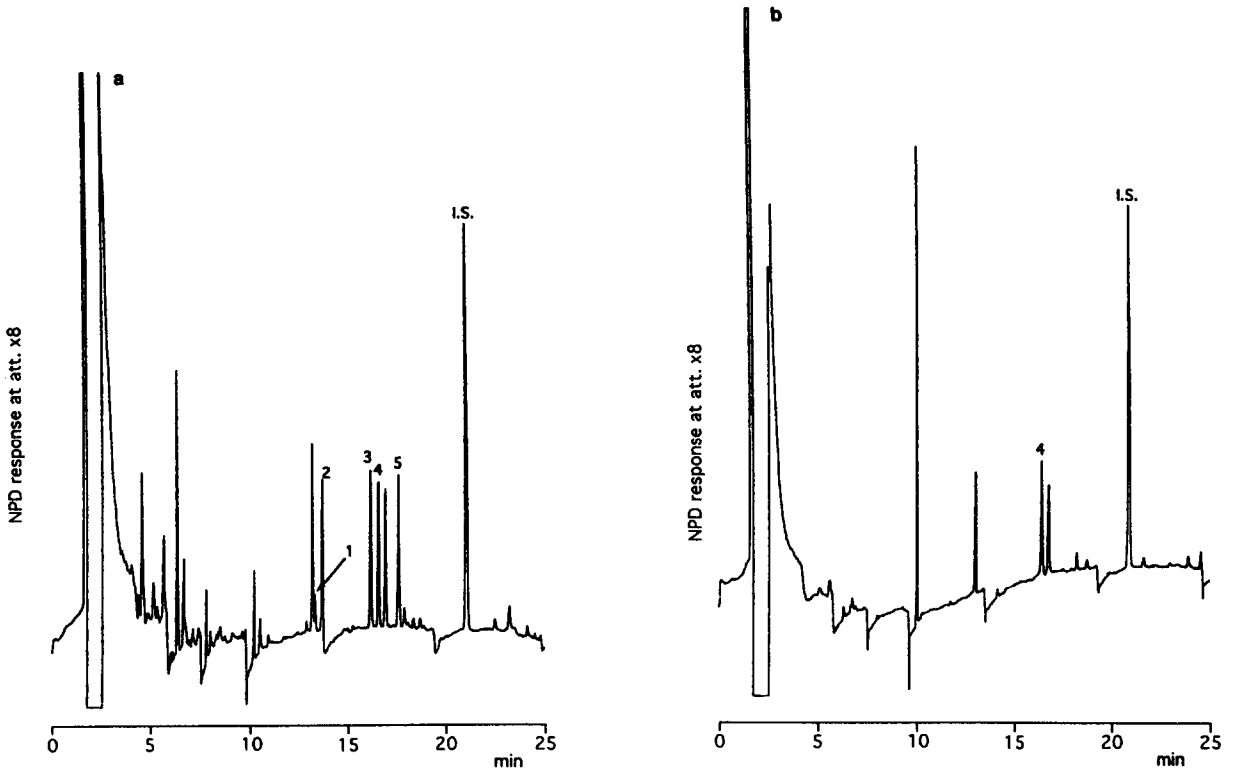

Fig. 1. GC–NPD chromatograms of (a) triazines spiked on peat soil and (b) atrazine in a field sample treated with atrazine. Peaks: 1 = desisopropylatrazine; 2 = desethylatrazine; 3 = simazine; 4 = atrazine; 5 = terbuthylazine; I.S. = internal standard. For chromatographic conditions see Experimental.

known that clay, with a higher content of organic carbon and another absorptive nature, will cause more irreversible binding of organic components than sand.

3.2. Linear model

The linear model could be applied on about 40 successful (recovery) experiments and was pri-

Table 3
Repeatability and linearity of SFE experiments

Repeatability/ linearity	Desisopropyl-atrazine	Desethyl-atrazine	Simazine	Atrazine	Terbuthyl-azine
Repeatability (series 3)					
Average (%)	9	32	50	66	66
S.D. (%)	15	21	21	8	7
R.S.D. (%)	165	65	43	12	11
Linearity (series 5)					
Average (%)	97	92	99	95	92
S.D. (%)	11	9	5	6	5
R.S.D. (%)	11	10	5	6	5

marily calculated using all ten parameters. The results showed that the addition of premixed modifier, both methanol and acetone, had only a small amount of relevant data points and had no significant effect and therefore, these parameters were excluded from the model.

Of the remaining parameters both the amount of modifier and the volume of the cell appeared to have a significant effect. However, the combination of these two parameters as the amount of modifier added per ml of cell volume seems logical. Therefore the quotient of the amount of modifier and the cell volume was introduced as an independent parameter in the linear model. With this new factor, the quality of the model improved and both the significant influence of the original parameters (amount of modifier and cell volume) vanished.

The coefficients resulting from the model calculation and the standard errors in these coefficients are shown in Table 4. The value of each coefficient can be interpreted as the effect that is calculated when the setting of the corresponding parameter is changed from the minimum into the maximum value. The standard error is the estimated standard deviation of this effect. The effect is expected to be proportional at smaller changes of the parameter settings, since it is a linear model.

3.3. Effects of parameters

From Table 4 can be concluded that the influence of the pressure on the recovery is very important. Recoveries increase when the pressure is increased in the range from 15 to 50 MPa. The amount of modifier added to the cell with respect to the volume of the cell is another important parameter. This influence however is not equal for all components, but increases with increasing component polarity. The influence of the temperature is small and negative. The negative contribution would, in combination with the positive contribution by an increase in pressure, point to a positive effect of an increase in density.

The influence of the amount of spike is significant for the first two components only. This effect may be artificially caused by the fact that a small amount of spike might not be detected at all ($<$LOD) and therefore a value of zero is included into the data set, while by a higher spike concentration and a similar recovery the real value (>0) can be used.

Variations in the recovery caused by type of soil (compared to sand) vary between -23 and 28%. These effects are relatively small compared to the effects of changing some other extraction parameters. Surprisingly, the effect is positive for the different triazines in peat, while a decrease in recovery should be expected on behalf of the better binding of the analytes to the soil matrices with higher contents of organic carbon in comparison with sand, as was seen for clay [12].

The effect of enlarging the extraction time is calculated by using the extraction time divided by the cell volume as the descriptive parameter. For all components a small (non-significant) negative effect is found. This suggests that the extraction time can be reduced without losing recovery.

The calculated values for the constant represent the predicted recovery when all parameter values are set to zero. For these non-optimal settings hardly any recovery is predicted for the more polar components —the metabolites desisopropylatrazine and desethylatrazine— increasing to a recovery of nearly 60% for the non-polar ones.

The residual standard deviations (difference between the experimental recoveries and the recoveries from the linear model) range between 15 and 25%. Comparing these standard deviations with the linearity experiment above, the latter appeared to be smaller. This is not surprising since the linearity was studied in a single series of experiments and using the same type of soil. So, inter-series variations and possible model imperfections are excluded.

Concluding, starting from these effects of the individual parameters to optimize the extraction parameters for triazines from soil, extractions have to be performed at maximum pressure (50 MPa), maximum addition of methanol as modifier (100 μl/ml cell volume), minimum tempera-

Table 4
Maximum effect of different parameters on the recoveries of individual triazines on basis of linear model

Parameter	Desisopropylatrazine		Desethylatrazine		Simazine		Atrazine		Terbuthylazine	
	Coefficient	Standard error	Coefficient	Standard error	Coefficient	Standard error	Coefficient	Standard error	Coefficient	Standard error
Constant	1	13	−3	12	14	19	48	19	65	20
Clay (1 = clay, 0 = sand or peat)	−21	6	−2	6	−4	9	−13	9	−23	10
Peat (1 = peat, 0 = sand or clay)	−16	9	7	8	28	12	28	13	23	13
Pressure (0 = 15 MPa, 1 = 50 MPa)	68	15	101	13	76	21	47	22	36	23
Temperature (0 = 31°C, 1 = 100°C)	−16	11	−42	10	−33	15	−28	16	−25	17
Spike volume (1 = 400 μl)	45	18	42	19	10	27	3	28	3	29
Extraction time/cell volume (0 = 6 min/ml, 1 = 11 min/ml)	−22	12	−15	12	−12	18	−9	19	−13	19
Modifier/cell volume (1 = 100 μl/ml)	107	15	100	15	128	22	77	23	49	24
Residual standard deviation	16.1		14.7		23.4		24.1		25.4	

See text. Residual standard deviation is the difference between the experimental recoveries and the recoveries from the linear model.

ture (50°C) and probably a lower extraction time, within the range of extraction conditions of this model. A test of these conditions has to be performed in a closer investigation.

3.4. Combined models

The results in Table 4 show a large amount of similarity between the models of the triazines studied. This in combination with the molecular familiarity between the triazines led to an attempt to describe the recoveries of all components using one model. An exception was made for both the constants and the influence of the amount of modifier/cell volume since these parameters show a large variation in effect between the different components. The result of this combined analysis is shown in Table 5. Note that the standard errors in the other coefficients decreased by more than a factor of two, due to the larger amount of data points in the model with respect to the separate models. The residual standard deviations are hardly changed while the number of overall model parameters is reduced from 40 to 16.

The application of a linear model without interactions is not a priori justified. To check the effect of non-linearities, the quadratic terms of both pressure and amount of modifier/cell volume were added to the model. These parameters had no significant influence. The separate effects of pressure and modifier/cell volume are the most important, therefore the interaction is determined. Introducing a combined effect of pressure and modifier/cell volume, the significance of the separate parameters disappears. This implies, that an increase of both parameters simultaneously will have an influence on extraction results, which is even larger than the effect predicted by the linear model.

Although no significant non-linear effects were found, the linear model has some intrinsic limitations since non-realistic recoveries below 0 and above 100% can be predicted easily. An elegant alternative is the use of a transformation which has these limits as asymptotic values. The application of some transformations is discussed by

Table 5
Effect of the parameters on the recovery of the triazines using a combined linear and a sigmoid model

Parameter	Combined linear model		Sigmoid model
	Coefficient	Standard error	Coefficient
Constant desisopropylatrazine	−4	9	−45
Constant desethylatrazine	7	9	−12
Constant simazine	23	9	5
Constant atrazine	46	9	56
Constant terbuthylazine	53	9	66
Clay (1 = clay, 0 = sand or peat)	−12	4	−21
Peat (1 = peat, 0 = sand or clay)	14	5	35
Pressure (0 = 15 MPa, 1 = 50 MPa)	65	9	144
Temperature (0 = 31°C, 1 = 100°C)	−28	6	−71
Spike volume (1 = 400 μl)	20	11	30
Extraction time/cell volume (0 = 6 min/ml, 1 = 11 min/ml)	−14	7	−37
Modifier/cell volume desisopropylatrazine (1 = 100 μl/ml)	84	11	149
Modifier/cell volume desethylatrazine (1 = 100 μl/ml)	74	11	113
Modifier/cell volume simazine (1 = 100 μl/ml)	75	11	198
Modifier/cell volume atrazine (1 = 100 μl/ml)	42	11	63
Modifier/cell volume terbuthylazine (1 = 100 μl/ml)	26	11	32
Residual standard deviation	21.5		19.9

Bourguignon et al. [13] to describe the effect of pH on HPLC retention.

In this paper a first attempt was made using the sigmoid function $S(y)$ for the transformation.

$$S(y) = [1 + \exp(-4y)]^{-1}$$

The results of the linear model, $M(x,p)$, were introduced in the sigmoid function. In order to enable a comparison with the linear model the sigmoid model $M_S(x,p)$ was scaled as:

$$M_S(x,p) = S[M(x,p)/100 - 0.5]100$$

The division and multiplication with 100 were necessary since all recoveries are expressed in percentages. A characteristic of the transformation is that values near 50% are not changed. The effects of most parameters are slightly larger compared with the results from the linear combined model. This indicates that for recoveries near 50% the influence of the parameters is larger than the calculated value from the linear model. Near the limits of 0 and 100% the influence of the parameters will be (much) smaller.

Concluding, the results of these two models confirm the effects obtained by the original linear model, the effects are larger and the errors are smaller. It delivers an interesting complementation, but no replacement of the linear model.

3.5. Field samples

In the series of experiments some field samples have been involved, which have been treated with atrazine. In Table 6, the atrazine concentrations for sample B1B (location Bergeijk; pitcode 1; second sampling), extracted eight times under different conditions, are shown. From this results can be seen, as for the spiked samples, that the concentrations are strongly varying with the extraction conditions from <9 to 50 μg/kg soil. Especially, the extractions with premixed modifiers score much lower than with modifier added directly to the extraction cell. The atrazine concentrations were corrected for recoveries as predicted by the linear model, resulting in a best estimation of the atrazine concentration of 41 μg/kg for this field sample. In combination with the average recovery of 61%, the variation in results is in agreement with the error predicted by the linear model. In Fig. 1b, a chromatogram of a field sample with atrazine is shown.

In Table 7, an overview is given for all field

Table 6
Atrazine in a field sample (B1B) using different SFE conditions

Series/ experiment No.	Cell volume (ml)	Modifier	Pressure (MPa)	Temperature (°C)	Extraction time (min)	Atrazine (μg/kg) determined	Atrazine[a] (μg/kg) after correction
2.6	10	300 μl MeOH	20	70	60	26	42
4.7	10	300 μl MeOH	50	50	70	29	27
6.7	10	CO_2–MeOH (90:10)	30	80	70	9	19
7.7	3.5	CO_2–MeOH (95:5)	25	90	30	12	37
8.1	10	300 μl MeOH	20	50	60	50	71
9.7	3.5	100 μl MeOH	30	100	30	28	49
10.8	10	CO_2–acetone (90:10)	30	100	70	17	44
11.6	3.5	CO_2–acetone (90:10)	20	70	30	<9	<26
				Average (7 experiments)		25	41
				S.D.		14	17
				R.S.D. (%)		56	40

[a]Corrections were calculated with the predicted recoveries according to the linear model.

Table 7
Atriazine concentrations (μg/kg) in field samples (series 8)

Sample code	Location	Sampling June 1991		Sampling October 1991	
		LLE	SFE	LLE	SFE
		June 1991	April 1992	October 1991	April 1992
B1A/B1B	Bergeijk	110	30	40	50
B2A/B2B	Bergeijk	100	22	63	37
L1A/L1B	Laren	100	59	33	41
L2A/L2B	Laren	130	48	50	41

Concentrations are not corrected for recovery and dry mass.

samples, which are collected at two locations (Bergeijk and Laren) at two pits (code 1 and 2) in two time periods (code A and B) after treatment with atrazine, in comparison with the results from conventional solvent extraction. Because the period of analysis is different for both techniques, only qualitative explanations can be given. The LLE concentrations, directly after treatment, are the highest, while four months later they are reduced with more than 50% by breakdown —although no metabolites could be determined— or irreversible binding to the matrix. The SFE of the first and second collection show comparable results, independent if samples are taken directly after treatment or later (and samples were stored in the laboratory in the dark at 4°C), and these values are in agreement with the results from the second solvent extraction.

4. Conclusions

The effects of various parameters on SFE of triazines from soil were tested using an experimental design, based on multiple linear regression. The model was adapted to exclude non contributing parameters and to combine some parameters to avoid interactions.

Two important parameters on the SFE efficiency were found, the pressure and the amount of modifier with respect to the cell volume. The recovery is increasing with increasing pressures, at the same time a small negative contribution was found for the temperature, both pointing towards a positive effect of an increase in density. The influence of the modifier is also essential, but only when it was added directly to the extraction cell and the effect is increasing with component polarity. Small effects were found for the type of soil and the extraction time. Residual standard deviations of the linear model range between 15 and 25%, whereas the intra-series repeatabilities are moderate (standard deviation of 5 to 11%), if optimal extraction conditions are chosen.

Starting from these effects of the individual parameters, extraction of triazines from soil can be optimized. Extractions have to be performed at maximum pressure (50 MPa), maximum addition of methanol as modifier (100 μl/ml cell volume), minimum temperature (50°C) and probably a shorter extraction time, within the range of extraction conditions of this model.

Two other models were tested, which make one data set for all triazines and apply a sigmoid transformation. The results of these two models give a more pronounced effect of the individual parameters.

A series of field samples, treated with atrazine, were involved in the experiments. The atrazine concentration can be determined with an error, which is in agreement with the error predicted by the linear model and further, SFE yields comparable results with solvent extractions.

Concluding, a dedicated experimental design and application of multiple linear regression

enabled a study of the effects of the individual parameters in supercritical fluid extraction. It is possible to reduce the number of experiments, to facilitate the evaluation of data and to distinguish possible interactions between several parameters.

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