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Journal of Chromatography A, 1072 (2005) 117–125
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

DDT degradation during enhanced solid–liquid extractions A consideration

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Abstract

Analysis of *p,p′*-DDT in solid environmental samples, like soil or sediment, requires monitoring the breakdown of this insecticide during sample preparation and measurement, which produces mainly *p,p′*-DDD and *p,p′*-DDE. The occurrence of matrix-enhanced *p,p′*-DDT degradation during GC injection is a well-known phenomenon; careful cleaning and pre-treatment of the GC injection port enables for an overcoming of this problem. Modern solid–liquid extraction methods apply high temperatures and/or pressures to enhance the extraction kinetics and diffusion rates of the target compounds from the solid matrix. Due to the parameters, high temperature and catalytic surface, DDT could break down during enhanced solid–liquid extraction comparable to the degradation process during GC injection. In the present study a performance evaluation by an experimental design of microwave-assisted extraction in closed vessels, pressurized-liquid extraction and fluidized-bed extraction with standard solutions of *p,p′*-DDT and with a quality control material was carried out. In order to identify the effect of matrix-enhanced DDT degradation during solid–liquid extraction a careful analytical protocol using isotope labelled standards was developed. At high temperatures matrix-enhanced degradation could be observed during all three investigated extraction methods. This result might have important implications on the resultant interpretations of environmental degradation of DDT.

Keywords: Degradation; Microwave-assisted extraction; Pressurized-liquid extraction; Fluidized-bed extraction; *p,p′*-DDT

1. Introduction

Since the 1940s the insecticide *p,p′*-DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] was heavily used in agriculture and for control the spread of vector-borne human diseases, especially typhus and malaria [1]. Due to its persistence and lipophilic nature, DDT tend to bioaccumulate in flora and fauna, and was, therefore, banned in many countries in the early 1970s [2], although it is used even today in some regions.

In general, the ratio of DDT and its main metabolites *p,p′*-DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethene] and *p,p′*-DDD [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane] are used to infer inputs of old versus new DDT residues or to attempt to characterize the importance of various environmental degradation pathways [3]. The DDT family also has been implicated in various aspects of endocrine disruption, with the degradation products more effective than the parent DDT in disrupting some hormonal pathways [4,5]. Therefore, an accurate analysis of the DDT species is mandatory for an understanding of their transport, fate, and ecological impact.

Unfortunately, DDT is known as fragile at high temperatures and shows matrix-enhanced degradation during GC injection [6]. Due to a contamination of the injection port with high boiling residues by sample injections over a sequence of routine GC analyses, an increase of *p,p′*-DDT degradation can be observed. GC degradation is indicated by formation of the degradation products *p,p′*-DDD via dechlorination and/or *p,p′*-DDE via dehydrochlorination. In order to monitor the breakdown, an injection and measurement of a performance evaluation standard containing only *p,p′*-DDT, but not *p,p′*-DDE or *p,p′*-DDD, at regular intervals throughout an analytical sequence is required, as demanded by US Environmental Protection Agency (EPA) Methods 508 [7] and 525.2 [8]. EPA Methods 8081A [9] and 8270C [10] limit GC-derived

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doi:10.1016/j.chroma.2005.03.024

breakdown of *p*,*p*′-DDT to not exceed 15% prior to calibration and also require a re-check using the performance evaluation standard for each 12 h shift. Otherwise, the 'injector maintenance and recalibration should be completed' before proceeding with additional sample analyses. The breakdown in percent can be calculated using the peak areas A following Eq. (1) as reported by Foreman and Gates [6]:

$$\%\ p,\ p'\text{-DDT} - \text{breakdown} = \frac{A_{p,p'\text{-DDD}} + A_{p,p'\text{-DDE}}}{A_{p,p'\text{-DDT}} + A_{p,p'\text{-DDD}} + A_{p,p'\text{-DDE}}} \times 100 \quad (1)$$

Modern solid–liquid extraction methods for environmental samples, like microwave-assisted extraction (MAE), fluidized-bed extraction (FBE) or pressurized-liquid extraction (PLE), apply high temperatures and/or pressures to enhance the extraction kinetics and diffusion rates of the target compounds from the solid matrix. Obviously, *p*,*p*′-DDT breakdown during enhanced solid–liquid extraction can be assumed, since comparable reaction conditions to GC injection are applied. The soil matrix can act as catalytic surface similar to the high boiling residues in a dirty GC injection port and the extraction temperatures, even much lower then during evaporation in the GC injector, are applied for several minutes. The combination of catalytic surface, temperature and hold-up time could lead to *p*,*p*′-DDT breakdown during extraction.

In the present study DDT-degradation during MAE, FBE and PLE has been investigated. In order to identify the effect of matrix-enhanced DDT degradation during solid–liquid extraction a careful analytical protocol using isotope labelled standards was developed.

2. Experimental

2.1. Reagents and chemicals

n-Hexane and acetone were supplied by Baker (Deventer, The Netherlands) (purity: Ultra resi-analyzed). *n*-Nonane (purum, 99%) was acquired from Fluka (Buchs, Switzerland). Dichloromethane and methanol were purchased from Promochem (Wesel, Germany; purity: Pico grade, for residue analysis). Silica gel (particle size 0.063–0.200 mm, for column chromatography, water content: 2.62%) and sodium sulfate anhydrous were obtained from Merck (Darmstadt, Germany). Diatomite (filter agent) was purchased from Aldrich (Gillingham, UK).

Reference compounds were supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany) as standard solutions: polychlorinated biphenyl (PCB) 209 (10 ng μL^{-1}) in cyclohexane; *p*,*p*′-DDE, *p*,*p*′-DDD and *p*,*p*′-DDT as pesticide mix in cyclohexane (10 ng μL^{-1} each); and isotope labelled $^{13}C_{12}$–*p*,*p*′-DDT as well as $^{2}H_{8}$–*p*,*p*′-DDE in acetone (100 ng μL^{-1}). The both isotope labelled standards were diluted in *n*-hexane to a final concentration of 10 ng μL^{-1}.

Nitrogen (purity: 99.9990%) and helium (purity: 99.9990%) were purchased from Air Liquide (Graz, Austria).

2.2. Sample

Optimization experiments were performed using a spiked river sediment as quality control material (qcm). For the production of the qcm sediment samples from the Yangtse river (Eastern China), available from a finished European Union project [11], were combined. In order to achieve sufficient intermixing, 5 kg of the pooled raw sediment were weighed into a 10 L glass bottle, well packed into a blending machine and rotated for 4 h. Thereafter, the sediment material was wetted with 2.5 L methanol and rotated for 1 h. This slurry was spiked with the analytes and intermixed for further 5 h. Afterwards, the mixture was transferred into an open container and, in order to evaporate the methanol, placed for 3 days into a hood at room temperature. After the material had been dried, it was ground, sieved and transferred again into a 10 L glass bottle, rotated again for 4 h over head and stored at a dry, dark place outside the laboratory for one year. Finally, the material was simply tested on homogeneity and characterized by means of Soxhlet extraction, as indicated later.

Additional experiments were performed with the reference material 'Sediment S37', produced according to the general principles for candidate reference material production except the final certification. Sediment S37 was tested on homogeneity within and between bottles and stability for several organochlorine pesticides over a time-course of 7 month by isotope-dilution GC–MS [12]. Furthermore, an organochlorine pesticides containing mallow powder TP29 [13] was analyzed for comparison purposes.

2.3. Soxhlet extraction

For definition of a reference procedure a 24 h Soxhlet extraction method was chosen. Five grams qcm, 5 g TP29 or 10 g sediment S37 were transferred into a cellulose extraction thimble (MN 645, 30 mm × 100 mm, Macherey-Nagel, Düren, Germany), 100 ng (10 μL) of the internal standards PCB 209, [$^{2}H_{8}$]*p*,*p*′-DDE and [$^{13}C_{12}$]*p*,*p*′-DDT were added, covered with glass wool (preconditioned for 72 h at 300 °C) and inserted into a 100 mL Soxhlet extractor. The samples were extracted under reflux with 150 mL of *n*-hexane–acetone (4:1, v/v) for 24 h. Since extraction thimbles are a potential contamination source, they were pre-extracted before use under reflux in 100 mL Soxhlet extractors with 150 mL *n*-hexane-acetone (1:1, v/v) for 4 h. Following extraction, the extracts were rotary evaporated to approximately 1 mL.

2.4. Microwave-assisted extraction

MAE experiments were carried out with a Multiwave3000 microwave oven (Anton Paar Physica, Graz, Austria) equipped with a 16-sample tray. Portions of 5 g (qcm

or TP29) or 10 g (sediment S37) were weighed into 100 mL perfluoroalkoxy (PFA) polymer extraction vessels equipped with polytetrafluoroethylene (PTFE)-sealed lip-tight caps and polyetheretherketone (PEEK) liners. The extraction solvent as indicated by the experimental design, 100 ng of each internal standard and magnetic stirring bars were added. The extraction was performed in temperature controlled mode, the temperature was ramped for 2 min. The maximum pressure increase rate was set at 80 kPa s^{-1}; the maximum pressure in the extraction vessels was programmed to not exceed 2 MPa. Temperature and hold-up time were set according to the experimental design. After a 15 min cooling step, the stirring bar was removed and rinsed with *n*-hexane. The raw extracts were separated from the solids by centrifugation at 100 × *g* for 3 min and transferred by means of a Pasteur pipette into 50 mL pear-shaped flasks. The remaining solid was washed three times with 1 mL portions of *n*-hexane and again separated by centrifugation. The combined solution of raw extract and washing solution was concentrated to nearly 1 mL.

2.5. Pressurized-liquid extraction

The PLE experiments were performed using an ASE 100 system (Dionex, Vienna, Austria), equipped with 11 mL stainless-steel extraction cells. The extraction cells were prepared as follows: the bottom of the extraction cell was covered with a cellulose filter (I.D. 16.2 mm, Schleicher&Schuell, Dassel, Germany) and about 0.5 g Bulk Isolute Sorbent (International Sorbent Technology, UK) as filter agent to prevent frit blockage or fine powder breakthrough into the collection bottle. Afterwards, 5 g (qcm or TP29) or 10 g sediment S37 were transferred into the extraction cell, which had been mixed with 1 g (S37) or 2 g (qcm and TP29) filter agent to improve the permeability. One hundred nanograms of each internal standard were added directly to the sample and, finally, the extraction cell was filled up with 0.5 g filter agent. The samples were extracted with *n*-hexane-acetone (1:1, v/v) in two extraction cycles under the conditions given by the experimental design as listed in Table 1. The experimental variables for the optimization were extraction temperature and static extraction time. Further PLE parameters were selected according to the default settings: The maximum extraction pressure was set to not exceed 1700 psi (=11.72 MPa); the flush volume was 60% of the extraction cell volume and the purge time was set to 1 min. The raw extracts were transferred into 100 mL pear-shaped flasks and concentrated to about 1 mL by means of a rotary evaporator.

2.6. Fluidized-bed extraction

For the extraction experiments a fexIKA variocontrol series extractor (IKA-Labortechnik, Staufen, Germany) was

Table 1
Design matrices for the three surface response designs with a three-fold repetition of the centre for the investigated extraction methods

Run	FBE			PLE		MAE		
	Number of cycles	Hold-up time (min)	*n*-Hexane	Hold-up time (min)	Temperature (°C)	Extraction time (min)	Temperature (°C)	Acetone
1	8	7	1	9	80	45	150	0.4
2	8	5	0.6	5	115	45	115	0.2
3	8	7	0.2	1	150	45	115	0.6
4	14	3	1	9	150	25	115	0.4
5	14	5	0.2	1	115	25	115	0.4
6	14	5	1	9	115	5	115	0.6
7	8	5	0.6	5	80	25	150	0.2
8	8	3	0.6	5	115	5	115	0.2
9	2	7	0.2	1	80	25	150	0.6
10	2	3	0.2	5	115	5	150	0.4
11	2	3	1	5	150	5	80	0.4
12	2	5	0.2			25	80	0.6
13	14	7	1			25	115	0.4
14	8	5	0.2			25	80	0.2
15	2	5	1			45	80	0.4
16	14	5	0.6			45	150	0.4
17	14	7	0.2			45	115	0.2
18	14	7	0.6			45	115	0.6
19	8	7	0.6			25	115	0.4
20	8	5	1			5	115	0.6
21	2	7	0.6			25	150	0.2
22	2	7	1			5	115	0.2
23	8	5	0.6			25	150	0.6
24	2	3	0.6			5	150	0.4
25	2	5	0.6			5	80	0.4
26	14	3	0.6			25	80	0.6
27	8	3	0.2			25	115	0.4
28	14	3	0.2			25	80	0.2
29	8	3	1			45	80	0.4

used. With the standard configuration of this system, four extractions could be simultaneously carried out. The system was equipped with a temperature sensor for an accurate setting of the heating and cooling temperature. For the extraction of solids each extraction tube was prepared with a fresh PTFE filter (47 mm I.D., 10–20 μm, fexIKA). Five grams TP29 or qcm or 10 g sediment S37 were weighed into an extraction tube and mixed with 3 g diatomite to enhance the permeability of the solid bed. One hundred nanograms of each internal standard were added and the specimens were extracted with 60 mL of an *n*-hexane–acetone mixture. The heating temperature was maintained at 90 °C for the first cycle in order to heat up the instrument and at 85 °C for the following cycles. The cooling temperature was set to 20 °C. Extraction solvent, static extraction time and number of extraction cycles were set according the experimental design. After extraction the raw extracts were transferred into 100 mL pear shaped flasks and the extracts were concentrated to about 1 mL by means of a rotary evaporator.

2.7. Clean up

Cartridges were prepared by weighing in 1 g silica gel and 3 g Na_2SO_4 each into empty 6 mL glass extraction cartridges (8 mm I.D.), which were equipped with polyethylene frits at the bottom. The solid bed was conditioned with 30 mL *n*-hexane and packed by a stream of N_2. The concentrated raw extract as well as 1 mL *n*-hexane–dichloromethane (7:3, v/v) solution from rinsing of the sample flask were injected on top of the column. After the analytes settled into the top 2–3 cm of the column, they were eluted from the column with further 11 mL *n*-hexane–dichloromethane (7:3, v/v) and collected in a 50 mL pear shaped flask. Two hundred and fifty microliters of *n*-nonane was added and the clean solution was concentrated by means of a rotary evaporator to about 1 mL. Thereafter, the eluate was further concentrated by a gentle stream of dry nitrogen to nearly 250 μL and transferred into GC autosampler vials for measurement.

2.8. Experimental design

Surface response designs, either 3^2 (PLE) or 3^3 for FBE and MAE, with a three-fold repetition of the center resulting in 11 (PLE) or 29 (FBE and MAE) experiments, were chosen for the optimization of the three extraction methods and the investigation of the matrix-enhanced DDT degradation. The complete design matrices are given in Table 1. For statistical calculations (to create experimental design and analyze the experimental results) the software package Statgraphics Plus version 3 for windows (Manugistics, Rockville, USA) was used.

2.9. GC–MS analysis

GC–MS Determination was carried out using a Hewlett-Packard (Waldbronn, Germany) HP6890 gas chromatograph equipped with an HP7683 auto sampler and a splitless injector (purge delay: 0.60 min; purge flow: 39.0 mL min^{-1}). The injector was equipped with a single tapered glass insert packed with a small amount of pesticide grade glass wool (Supelco, Bellefonte, PA, USA) and maintained at 250 °C, 1 μL of each sample was injected. The capillary column used was a HP-5MS, 30 m × 250 μm I.D. and 0.25 μm film thickness. The carrier gas was helium at a constant flow rate of 1.1 mL min^{-1}. The temperature program was: 70 °C for 0.50 min ramped to 170 °C at 25 °C min^{-1}, to 190 °C at 4 °C min^{-1}, to 230 °C at 10 °C min^{-1}, to 270 °C at 4 °C min^{-1}, to 300 °C at 30 °C min^{-1} and held at 300 °C for 5 min. The gas chromatograph was coupled to an HP5973 mass selective detector (electron impact: 70 eV) operated in SIM mode using the *m*/*z* values listed in Table 2. The interface temperature was maintained at 300 °C. The instrument was tuned daily with perfluorotributhylamine (PFTBA) using the Automatic Tune (ATUNE). Calibration was accomplished by internal standardization with PCB 209, [$^2H_8$]*p*,*p*′-DDE and [$^{13}C_{12}$]*p*,*p*′-DDT at six levels spanning the range 15–360 pg μL^{-1}. [$^{13}C_{12}$]*p*,*p*′-DDT was used as internal standard for *p*,*p*′-DDT; [$^2H_8$]*p*,*p*′-DDE and PCB 209 were used as internal standard for *p*,*p*′-DDE and *p*,*p*′-DDD. In addition, each calibration standard and sample extract was injected in duplicate. After 10 injections a standard solution containing *p*,*p*′-DDT and [$^{13}C_{12}$]*p*,*p*′-DDT at a concentration level of 100 pg μL^{-1} was injected to evaluate the performance of the system. Chromatographic peak areas were fitted by linear regression and the correlation coefficients ranged from 0.9992 to 0.9999.

Table 2
Ions selected for MS detection in SIM mode, the underlined ions were used as target ion; the second were used as qualifiers, compounds listed in sequence of elution

Analyte	Abbreviation	*m*/*z*
[$^2H_8$]*p*,*p*′-DDE	DDDE	254, 256
[$^{13}C_{12}$]*p*,*p*′-DDE	13CDDE	258, 260
p,*p*′-DDE	DDE	246, 248
[$^{13}C_{12}$]*p*,*p*′-DDD	13CDDD	247, 249
p,*p*′-DDD	DDD	235, 237
[$^{13}C_{12}$]*p*,*p*′-DDT	13CDDT	247, 249
p,*p*′-DDT	DDT	235, 237
PCB 209	PCB209	498, 500

3. Results and discussion

3.1. DDT-degradation during GC injection

Since it is well known, that DDT could break down during GC injection [6] a careful analytical protocol was chosen for the investigation of the DDT degradation during GC measurement. After not more than 10 injections a performance evaluation standard containing 100 pg μL^{-1} of *p*,*p*′-DDT as well as [$^{13}C_{12}$]*p*,*p*′-DDT was injected and the DDT breakdown in percent was calculated following Eq. (1). A DDT-

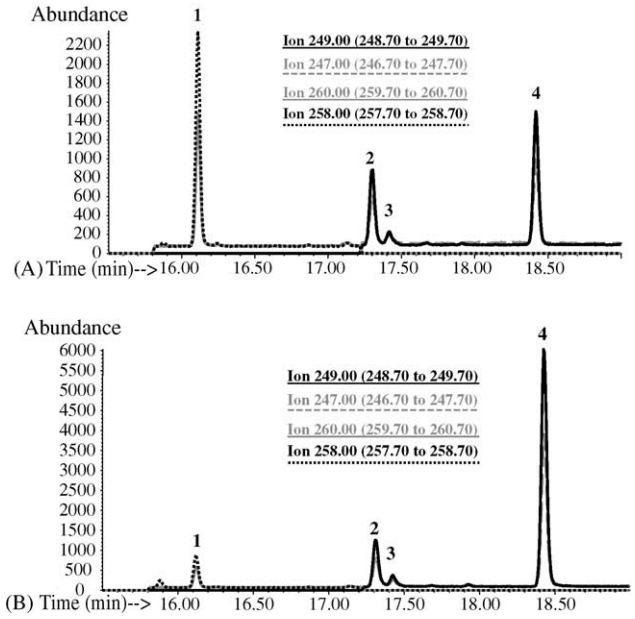

Fig. 1. Ion traces during mass spectrometric detection for [$^{13}C_{12}$]*p,p′*-DDT and the $^{13}C_{12}$-labelled metabolites after injection of [$^{13}C_{12}$]*p,p′*-DDT into a dirty GC glass insert (A) and a cleaned glass insert (B). Peak identification: (1) [$^{13}C_{12}$]*p,p′*-DDE; (2) [$^{13}C_{12}$]*p,p′*-DDD; (3) [$^{13}C_{12}$]*o,p′*-DDT; (4) [$^{13}C_{12}$]*p,p′*-DDT.

degradation rate greater than 10% was attributed to a dirty glass insert. Fig. 1 demonstrates clearly the matrix-enhanced DDT breakdown in the glass insert during GC injection.

The ion traces given in Fig. 1 are obtained after injection of 100 pg [$^{13}C_{12}$]*p,p′*-DDT into a dirty glass insert (A) and a cleaned glass insert (B). For both chromatograms the sum of the peak areas is nearly the same. Obviously, DDT is degraded mainly by dehydrochlorination leading to DDE as main degradation product. Based on this results and assuming that [$^{13}C_{12}$]*p,p′*-DDT behave similar to *p,p′*-DDT, [$^{13}C_{12}$]*p,p′*-DDT is the ideal internal standard for this compound. On the other hand, [$^{13}C_{12}$]*p,p′*-DDT is an inadequate internal standard for the both main degradation products *p,p′*-DDD and *p,p′*-DDE and the both $^{13}C_{12}$-labelled degradation products should be used alternatively. The aim of our study was, however, the investigation of the DDT-degradation, which would not have been possible when using all three $^{13}C_{12}$-labelled standard compounds. Therefore [$^{2}H_{8}$]*p,p′*-DDE or PCB 209 were used as internal standard for *p,p′*-DDE and *p,p′*-DDD.

3.2. Microwave-assisted extraction

The effect of the [$^{13}C_{12}$]*p,p′*-DDT-breakdown during the MAE experiment was evaluated using a similar statistical approach as for optimization. A 3^3 surface response design with a three-fold repetition of the center was chosen for investigation of the MAE system. Three experimental factors, identified as significantly influential during a previously published screening experiment [14], have been investigated, namely the percentage of acetone in the *n*-hexane-acetone solvent mixture, the extraction time as well as the extraction temperature.

Hypersurfaces were constructed from the response (Y) as a function of variable composition (x_i) using polynominals as they are expressed in Eq. (2)

$$Y = \beta_0 + \sum_i \beta_i x_i + \sum_{ij} \beta_{ij} x_i x_j \quad (2)$$

Y being the breakdown rates, x_i and x_j the variables considered for the optimization of the extraction and β_i and β_{ij} the parameters to be calculated. The most general function allowed for the 3^3 surface response designs is given in Eq. (3)

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{22} x_2^2 + \beta_{23} x_2 x_3 + \beta_{33} x_3^2 \quad (3)$$

with x_1 as the holding time after reaching the extraction temperature, x_2 as extraction temperature and x_3 as the volume fraction of acetone in the *n*-hexane–acetone solvent mixture. Eq. (4) is completed by the computationally obtained results for the parameters. Bold numbers indicate significant factors as identified by the analysis of variance (ANOVA) at the 95% confidence level.

$$Y = 67.339 - \mathbf{0.414} x_1 - \mathbf{0.545} x_2 - 0.812 x_3 + \mathbf{0.0001} x_1^2 + 0.003 x_1 x_2 + \mathbf{0.006} x_1 x_3 + \mathbf{0.002} x_2^2 + 0.001 x_2 x_3 + \mathbf{0.007} x_3^2 \quad (4)$$

As can be seen from the main effects plot for the breakdown of [$^{13}C_{12}$]*p,p′*-DDT (Fig. 2), higher degradation rates could be observed at longer extraction times and higher extraction temperatures. A minimum for the extraction temperature could be found around 110 °C. For the composition of *n*-hexane–acetone, a clear minimum for the breakdown was observed at 50 vol.% acetone. This clear finding of a *p,p′*-DDT-breakdown during microwave-assisted extraction indicates extraction temperatures of 110 °C or lower combined with extraction times of 20 min or shorter and the use of the azeotropic mixture of *n*-hexane–acetone (1:1, v/v) to prevent *p,p′*-DDT losses by degradation during MAE. Additionally, this results are correlated with the optimum settings for the extraction of *p,p′*-DDT as already published previously [14].

The pareto chart for the *p,p′*-DDT during MAE is given in the second part of Fig. 2. The pareto chart visualizes the influence of each factor on the result/degradation grouping the most influential factor at the top of the list. Standardized pareto chart shows each effect divided by its standard error. Factor bars exceeding the vertical significance line, exert a statistically significant influence on the result at the 95% confidence level (ANOVA). As expected, the most influential factor is the extraction temperature, but the static extraction time exceeds a statistical influence on the degradation, too. The calculated regression coefficient R^2 of 0.821 point out

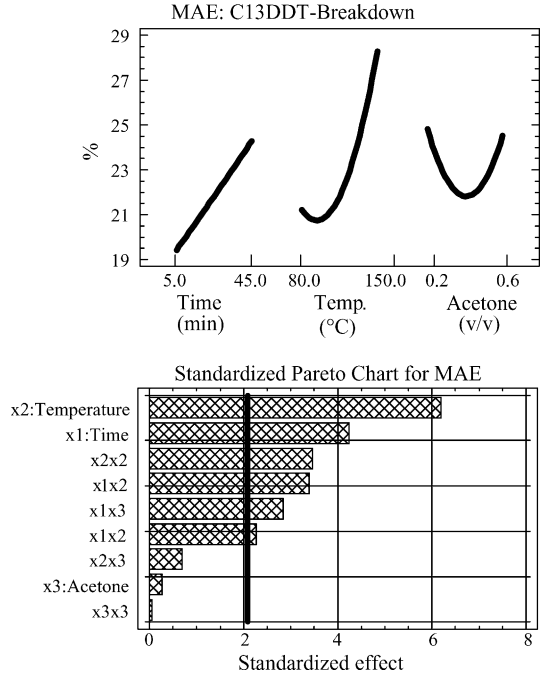

Fig. 2. Main effects plot and pareto chart of the 3^3 surface response design for the [$^{13}C_{12}$]*p*,*p*′-DDT degradation during MAE.

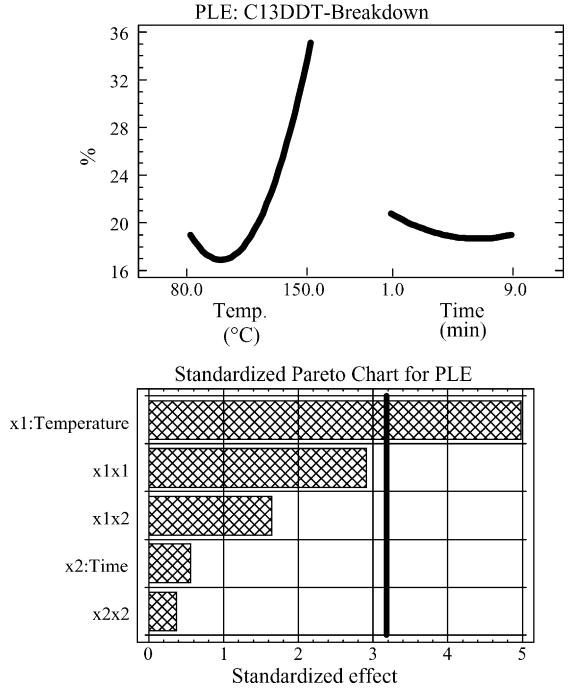

Fig. 3. Main effects plot and pareto chart of the 3^2 surface response design for the [$^{13}C_{12}$]*p*,*p*′-DDT degradation during PLE.

that the experimental results are explained by the factor effects studied.

Although numerous studies of MAE are published, the phenomena of matrix-enhanced *p*,*p*′-DDT degradation during MAE was not described. Several years ago, the group of Lopez-Avila studied the stability of organochlorine pesticides including *p*,*p*′-DDT during microwave-assisted extraction from solid samples [15,16]. Unfortunately, in these studies the compounds were grouped and the stability of the group was investigated. Nevertheless, at high temperatures (145 °C) the recovery was already reduced. Other studies on MAE optimization applied temperatures up to 130 °C [17] or used naturally contaminated soil samples with a very high *p*,*p*′-DDT background value and low values of the metabolites [18]. MAE experiments performed with pure [$^{13}C_{12}$]*p*,*p*′-DDT and *p*,*p*′-DDT standard solutions and free from solid material did not lead to DDT degradation.

3.3. Pressurized-liquid extraction

The effect of the pressurized-liquid extraction on the [$^{13}C_{12}$]*p*,*p*′-DDT degradation was evaluated by a 3^2 surface response design. Hypersurfaces were constructed from the response (*Y*) as a function of variable composition (x_i) using polynominals as they are expressed in Eq. (2). The most general function for the 3^2 surface response design is given in Eq. (5)

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{12} x_1 x_2 + \beta_{22} x_2^2 \qquad (5)$$

with x_1 as the extraction temperature and x_2 as the holding time after reaching the heating temperature. Eq. (6) is completed by the computationally obtained results for the parameters. Bold numbers indicate again significant factors as identified by the ANOVA at the 95% confidence level.

$$Y = 85.054 - \mathbf{1.210} x_1 - 3.809 x_2 + 0.006 x_1^2 + 0.013 x_1 x_2 + 0.313 x_2^2 \qquad (6)$$

The calculated regression coefficient R^2 for this experiment was 0.898, indicating that the both investigated factors extraction temperature and time explain the [$^{13}C_{12}$]*p*,*p*′-DDT degradation almost perfect. For visualization of the results a main effects plot and a pareto chart from the calculated factor effects were drawn.

As can be seen from the pareto chart in Fig. 3 only the extraction temperature was identified as statistically significant, although minima could be observed from the main effects plot for both factors. A minimum for the [$^{13}C_{12}$]*p*,*p*′-DDT degradation could be observed at 100 °C and estimated 7 min static extraction time, which is correlated with the optimum of a PLE optimization experiment for organochlorine pesticides [19]. Several studies investigated PLE for the extraction of organochlorine compounds – including *p*,*p*′-DDT – from various solid matrices [18,20–22]. Nearly all of them found the solvent mixture of *n*-hexane-acetone (1:1, v/v) as suitable for the extraction of organochlorine compounds, therefore, this mixture has been used for the presented experiments. Additionally, after extraction the *n*-hexane–acetone mixture can easily be evaporated applying rather low temperatures, which is an advantage for organochlorine pesticide analysis. The effect of *p*,*p*′-DDT degradation during PLE has not

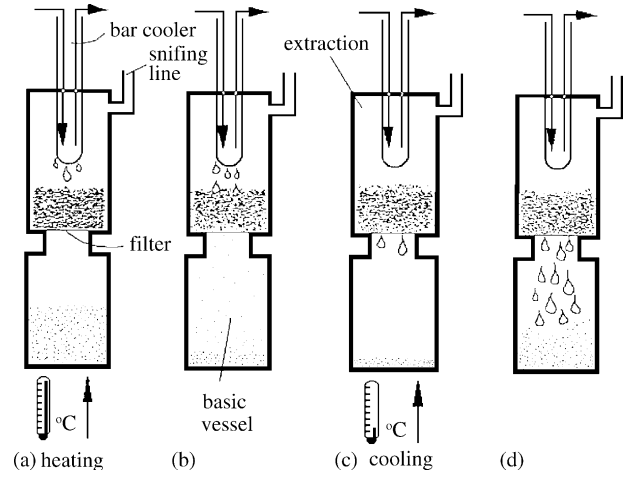

Fig. 4. Operating principle of FBE.

been described in detail yet, because in naturally contaminated samples the distribution between the mother substance *p,p′*-DDT and its metabolites is sometimes adverse [18] or problems in the sample clean-up occurred [21]. As could be shown from the results the effect of *p,p′*-DDT breakdown is strongly related to the extraction temperature. At high extraction temperatures – higher 130 °C – the effect increased up to a breakdown rate greater than 30%.

3.4. Fluidized-bed extraction

FBE is a Soxhlet-related extraction technique [23], but with the advantage of an extraction near the boiling point of the chosen extraction solvent. Thus, leads to a more effective kinetics and a faster extraction. The basic principle of the FBE technique is graphically displayed in Fig. 4.

The extraction solvent is filled into the basic vessel and the heating-cooling block of the device is heated up to a predefined temperature, which is selected according to the boiling point of the extraction solvent. The evaporated solvent penetrates the filter and condenses primarily at the high-efficiency condenser (a). The condensed solvent drips back into the extraction material/mixed solvent and is recollected there. The constant flow of solvent vapor from the basic vessel heats up and vigorously fluidizes the mixture. Extraction at elevated temperature and fluidizing agitation both account for particularly effective extraction kinetics (b). Following the process step ‘heating’ the solvent is recollected into the extraction tube. For this purpose the system is programmed to turn off heating and to simultaneously cool down the basic heating-cooling block as well as the solvent contained in the basic vessel (c). A vacuum is therefore produced in the basic vessel as a result of the quick cooling process and the resultant differential pressure transports the extract through the filter into the basic vessel (d). This process sequence may be cyclically repeated for any number of pre-programmed steps.

The effect of the FBE on the [$^{13}C_{12}$]*p,p′*-DDT degradation was evaluated by a 3^3 surface response design. Again,

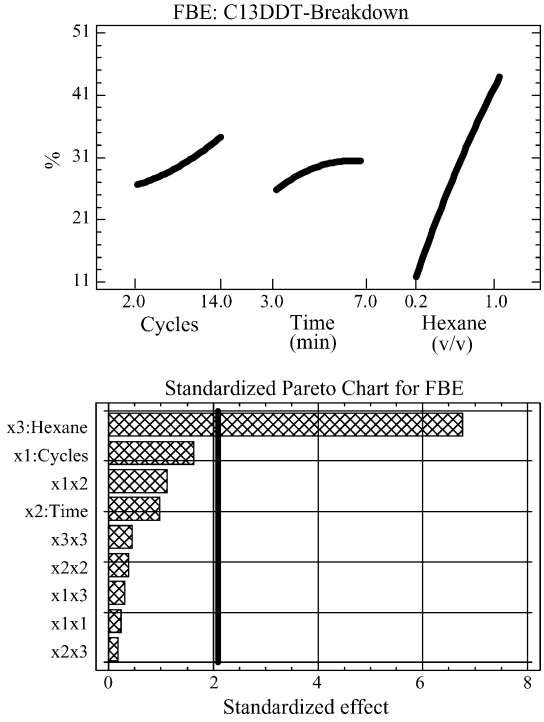

Fig. 5. Main effects plot and pareto chart of the 3^3 surface response design for the [$^{13}C_{12}$]*p,p′*-DDT degradation during FBE.

hypersurfaces were constructed from the response (Y) as a function of variable composition (x_i) using polynominals as they are expressed in Eq. (2). The most general function for a 3^3 surface response design has already been given in Eq. (3). Eq. (7) is completed by the computationally obtained results for the parameters for the factors number of extraction cycles (x_1), hold-up time after reaching the heating temperature (x_2) and volume fraction of *n*-hexane (x_3). Bold numbers indicate significant factors as identified by the analysis of variance (ANOVA) at the 95% confidence level.

$$\begin{aligned} Y &= -23.659 + 1.380x_1 + 6.483x_2 + \mathbf{46.764}x_3 \\ &\quad +0.025x_1^2 - 0.270x_1x_2 + 0.353x_1x_3 - 0.355x_2^2 \\ &\quad +0.612x_2x_3 - 10.476x_3^2 \end{aligned} \tag{7}$$

The calculated regression coefficient R^2 for this experiment was 0.818. For visualization of the results a main effects plot and a pareto chart from the calculated factor effects were drawn for the $^{13}C_{12}$–*p,p′*-DDT degradation.

As is apparent from the main effects plot (Fig. 5), higher degradation rates were observed for longer extraction times, more extraction cycles and greater amounts of *n*-hexane in the extraction solvent mixture. Pure *n*-hexane has the highest boiling point (69 °C) of the solvents investigated and it takes much longer to evaporate the solvent and produce a slurry of solvent and sample. It is probable that the sample is heated more or less dry for some time, which seems to increase the degradation of *p,p′*-DDT. These findings

Table 3
Sediment S37 [12] – results for MAE, PLE and FBE in comparison with Soxhlet extraction and the published values in ng g^{-1}, mean ± standard deviation (SD) and relative standard deviation (RSD) in %

Compound	Published value		Soxhlet		MAE		PLE		FBE	
	Mean ± SD	RSD	Mean ± SD	RSD	Mean ± SD	RSD	Mean ± SD	RSD	Mean ± SD	RSD
p,p′-DDE	2.33 ± 0.09	3.86	2.27 ± 0.09	3.93	2.29 ± 0.15	6.76	2.32 ± 0.12	5.17	2.26 ± 0.04	1.65
p,p′-DDD	1.29 ± 0.17	13.18	1.17 ± 0.08	6.22	1.22 ± 0.12	9.80	1.31 ± 0.11	8.40	1.29 ± 0.02	1.92
p,p′-DDT	3.42 ± 0.47	13.74	3.45 ± 0.10	2.90	3.35 ± 0.13	3.88	3.48 ± 0.09	2.59	3.46 ± 0.07	2.02

Table 4
Mallow powder TP29 [13] – results for MAE, PLE and FBE in comparison with Soxhlet extraction and the published values in ng g^{-1}, mean ± standard deviation (SD) and relative standard deviation (RSD) in %

Compound	Published value		Soxhlet		MAE		PLE		FBE	
	Mean ± SD	RSD	Mean ± SD	RSD	Mean ± SD	RSD	Mean ± SD	RSD	Mean ± SD	RSD
p,p′-DDE	14.03 ± 1.73	12.33	14.33 ± 0.72	5.02	16.08 ± 1.43	8.89	15.02 ± 0.63	4.19	14.28 ± 0.48	3.36
p,p′-DDD	11.33 ± 0.97	8.56	11.78 ± 0.51	4.33	11.90 ± 0.78	6.55	11.98 ± 0.58	4.84	11.63 ± 0.33	2.84
p,*p′*-DDT	25.43 ± 3.76	14.79	27.01 ± 1.49	5.52	23.78 ± 2.52	10.60	26.34 ± 2.10	7.97	26.88 ± 1.00	3.72

were in correlation with optimized conditions for FBE of organochlorine compounds [24] and very little [$^{13}C_{12}$]*p,p′*-DDT breakdown was observed during FBE operated under optimum conditions, which were 11 extraction cycles with 5 min static extraction time and an *n*-hexane–acetone (1:1, v/v) mixture. Additionally, when FBE experiments were performed with pure [$^{13}C_{12}$]*p,p′*-DDT and *p,p′*-DDT solutions free from solid material no degradation was observed.

3.5. Evaluation using reference materials

In order to verify the performance of the three investigated enhanced solid–liquid extraction methods for the extraction of *p,p′*-DDT and its both main metabolites *p,p′*-DDE and *p,p′*-DDD, two reference materials, mallow powder TP29 and sediment S37, were analyzed in triplicate. TP29 was chosen as representative of plant materials. This herb material contains *p,p′*-DDT as well as *p,p′*-DDE and *p,p′*-DDD in concentrations between 11 and 25 ng g^{-1}. S37, an environmental reference material, contains rather low concentrations of *p,p′*-DDT and its degradation products (in the range 1.3–3.4 ng g^{-1}). For each extraction method the parameter settings combination, which shows the lowest [$^{13}C_{12}$]*p,p′*-DDT degradation, were used. For comparison purposes, three samples of the both reference materials were also extracted by the conventional Soxhlet technique. The results for MAE, PLE, FBE and Soxhlet as well as the published values for the both different materials are listed in Tables 3 and 4.

The data obtained by the three investigated extraction methods MAE, PLE and FBE were in good agreement with those obtained by the classic Soxhlet extraction and the published values. Additionally, the best precision of all method was received by FBE, followed by Soxhlet, PLE and MAE. Based on this result all three methods are valid for routine DDT analysis of solid matrices when operated under the stated optimum conditions.

4. Conclusion

p,p′-DDT is less stable during analysis than proposed and a careful analytical protocol should be established to achieve correct and reliable results. As could be shown from the experiments, *p,p′*-DDT degradates matrix enhanced during GC injection as well as hot solid–liquid extraction. Therefore,

(1) Extraction parameters have to be optimised, especially the applied extraction temperature should not exceed 120 °C to avoid DDT break down.
(2) The glass insert of the GC injector has to be maintained carefully and after not more than 10 injections a pure DDT standard solution should be analysed to identify a dirty glass insert.
(3) The use of isotope-labelled internal standard, added prior the extraction, and GC–MS for quantification could solve the problem.

Acknowledgements

The authors thank Dionex Corp. (Vienna, Austria), Anton Paar GmbH (Graz, Austria) and IKA-Labortechnik (Staufen, Germany) for providing the extraction instruments used in this study.

References

[1] R.L. Metcalf, J. Agric. Food Chem. 21 (1973) 511.
[2] H.M. Bold, G.H. Degen, Arch. Toxicol. 76 (2002) 187.
[3] M. Zumbado, M. Goethals, E.E. Álvarez-León, O.P. Luzardo, F. Cabrera, L. Serra-Majem, L. Domínguez-Boada, Sci. Total. Environ. 339 (2005) 49.
[4] M. Patlak, Environ. Sci. Technol. 30 (1996) 210A.
[5] J.J.A. Mendes, Food Chem. Toxicol. 40 (2002) 781.
[6] W.T. Foreman, P.M. Gates, Environ. Sci. Technol. 31 (1997) 905.
[7] Methods for the Determination of Organic Compounds in Drinking Water, US Environmental Protection Agency, Office of Research and

Development, U.S. Government Printing Office, Washington, DC, 1995, EPA-600/R-95/131, method rev. 3.1, p. 508.1.
[8] Methods for the Determination of Organic Compounds in Drinking Water, U.S. Environmental Protection Agency, Office of Research and Development, U.S. Government Printing Office, Washington, DC, 1995, EPA-600/R-95/131, method rev. 2.0, p. 525.2.1.
[9] Test Methods for Evaluating Solid Waste: Laboratory Manual—Physical/Chemical Methods, SW-846, US Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, DC, 1995, v. IB, method rev. 1, p. 8081A 1.
[10] Test Methods for Evaluating Solid Waste: Laboratory Manual—Physical/Chemical Methods, SW-846, US Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, DC, 1995, v. IB, method rev. 1, p. 8270C 1.
[11] B.M. Gawlik, B. Platzer, H. Muntau, Monitoring of Chlorinated and Other Organic Trace Contaminations in Selected Stretches of the Liao and Yangtse River (China) European Commission EUR 19531, EN, Ispra, Italy, 2000.
[12] B.M. Gawlik, D. Martens, B. Henkelmann, K.-W. Schramm, A. Kettrup, H. Muntau, Fresenius J. Anal. Chem. 367 (2000) 232.
[13] H. Muntau, B.M. Gawlik, A. Sahuquillo, M. Bianchi, A. Rehnert, Accred. Qual. Assur. 9 (2004) 247.
[14] M. Gfrerer, E. Lankmayr, Anal. Chim. Acta, in press.
[15] V. Lopez-Avila, J. Benedicto, K.M. Bauer, J. AOAC Int. 81 (1997) 1224.
[16] V. Lopez-Avila, R. Young, W.F. Beckert, J. AOAC Int. 81 (1998) 462.
[17] I. Silgoner, R. Krska, E. Lombas, O. Gans, E. Rosenberg, M. Grasserbauer, Fresenius J. Anal. Chem. 362 (1998) 120.
[18] L.J. Fitzpatrick, J.R. Dean, M.H.I. Comber, K. Harradine, K.P. Evans, J. Chromatogr. A 874 (2000) 257.
[19] S. Chen, M. Gfrerer, E. Lankmayr, X. Quan, F. Yang, Chromatographia 58 (2003) 631.
[20] P. Suchan, J. Pulkrabová, J. Hajšlová, V. Kocourek, Anal. Chim. Acta 520 (2004) 193.
[21] E. Concha-Graña, M.I. Turnes-Carou, S. Muniategui-Lorenzo, P. López-Mahía, E. Fernández-Fernández, D. Prada-Rodríguez, J. Chromatogr. A 1047 (2004) 147.
[22] P. Popp, P. Keil, M. Möder, A. Paschke, U. Thuss, J. Chromatogr. A 774 (1997) 203.
[23] M. Gfrerer, B.M. Gawlik, E. Lankmayr, Anal. Chim. Acta 527 (2004) 53.
[24] M. Gfrerer, C. Fernandes, E. Lankmayr, Chromatographia 60 (2004) 681.