

Pressurized Liquid Extraction of Organochlorine Pesticides from Certified Solid Materials

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Abstract

In order to propose a versatile method to use in laboratories devoted to environmental analysis, a scheme for the determination of organochlorine pesticides from different solid matrices is evaluated. Pressurized liquid extraction is chosen as the sample preparation technique, followed by the purification of the extract by means of solid-phase extraction with Envi-carb cartridge (100 m²/g). Finally, the extracts are analyzed by programmed temperature vaporization (PTV)-gas chromatography (GC)-electron-capture detection and PTV-GC-mass spectrometry. The suitability of the method for the analysis of different matrices is determined by the extraction and analysis of four certified reference materials of solid matrices: CRM 804-050 (soil), SRM 1941b (organics in marine sediment), SRM 1944 (New York/New Jersey waterway sediment) and SRM 1649a (Urban Dust). Good statistical concordance between the results obtained and the certified or reference concentration are observed for most of the analyzed pesticides.

Introduction

Nowadays, the broad range of compounds and matrices analyzed by the laboratories devoted to environmental analysis makes versatile analytical methods mandatory to determine the compounds of interest in different matrices, avoiding the development of a different analysis method for each matrix. The toxicity and persistence of organochlorine pesticides, some of them carcinogenic (1) and endocrine disrupters (2), makes their monitoring very important.

The use of pressurized liquid extraction (PLE), has some advantages over other extraction techniques, such as short extraction time, low solvents consumption, no necessary filtration of the extract, and the universal use of solvents as well as the ability to programming different extraction cycles for a sample. These advantages make the PLE very useful for the analysis of organochlorine pesticides and other compounds from different matrices: solid wastes (3), soil (4–6), vegetation (7), fish (8), fruit

(9), vegetables (10), etc. Moreover, PLE is proposed as an extraction technique in the Environmental Protection Agency method 3545 for the analysis of organic compounds in solid matrices.

This article concerns the evaluation of a sample handling procedure for the environmental analysis of organochlorine pesticides. The analysis method involves pressurized liquid extraction, solid-phase extraction (SPE) clean up with carbon cartridges, and determination by large volume programmed temperature vaporization (LV-PTV) injection gas chromatography (GC) with electron-capture detection (ECD) and mass spectrometry (MS) detection. The chromatographic methods were proposed in previous works (11,12). The extraction method had been initially developed and optimized for the analysis of organochlorine pesticides from soils (6), and now the scope is to assay the same sample preparation method to analyze other complex solid matrices, such as sediments and urban dust. These matrices were selected because they are frequently analyzed in environmental monitoring laboratories and because of the availability of certified reference materials that contains organochlorine pesticides. The suitability of the proposed method for the analysis of different matrices was determined by the analysis of four certified reference materials of solid matrices containing a broad range of concentration (0.6–1500 µg/kg) of organochlorine pesticides: CRM 804-050 (soil), SRM 1941b (organics in marine sediment), SRM 1944 (New York/New Jersey waterway sediment), and SRM 1649a (urban dust).

Materials and Instruments

n-Hexane (95%), dichloromethane, and acetone super purity solvents were purchased from Romil (Cambridge, UK). Ethyl acetate for residue analysis was from Panreac (Barcelona, Spain).

Individual solid standards of *p,p'*-DDE, *o,p'*-DDD, *p,p'*-DDT, α -HCH, and heptachlor pestanal were supplied by Riedel-de-Haën (Seelze, Germany). Individual solid standards of hexachlorobenzene (HCB), *p,p'*-DDD, *o,p'*-DDE, α -chlordan, and γ -chlordan and internal standard 2,4,5,6-tetrachloro-*m*-xylene (TCMX) were supplied by Supelco (Bellefonte, PA). Standards of *cis*-nonachlor

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and *trans*-nonachlor (100 µg/mL) in hexane were also supplied by Supelco. Working standard solutions were prepared by dilution in *n*-hexane.

Certified reference material of Pesticides on Soil CRM804-050 was obtained from Resource Technology (Laramie, Wyoming). New York/New Jersey Waterway Sediment SRM 1944, Organics

in Marine Sediment SRM 1941b, and Urban Dust SRM 1649a were from the National Institute of Standards and Technology (NIST) (Gaithersburg, MD).

Diatomaceous earth acid washed not further calcined was from Sigma (Sigma-Aldrich Chemie GmbH, Germany). Copper metal powder purissimum from Panreac was used to reduce the presence of the sulphur from the extracts. It was activated with 2M hydrochloric acid (HCl). Envi-Carb Packing, 12 mL (1 g), 100 m²/g was supplied by Supelco.

Table I. MS Working Conditions in SIM Mode

| Set | Pesticides | t _R (min) | Quantitation ion | Confirmation ion |
|-----|-------------------------|----------------------|------------------|------------------|
| 1 | TCMX | 35.50 | 207 | |
| 2 | α-HCH | 36.78 | 181 | 219 |
| 2 | HCB | 37.15 | 284 | 249 |
| 3 | Heptachlor | 41.35 | 272 | 100 |
| 4 | <i>o,p</i> -DDE | 45.80 | 246 | 318 |
| 4 | γ-Chlordane | 46.40 | 373 | 377 |
| 4 | α-Chlordane | 46.60 | 373 | 377 |
| 4 | <i>trans</i> -Nonachlor | 46.85 | 407 | 409 |
| 4 | <i>p,p'</i> -DDE | 47.43 | 246 | 318 |
| 4 | <i>o,p</i> -DDD | 47.84 | 235 | 165 |
| 5 | <i>cis</i> -Nonachlor | 49.65 | 407 | 409 |
| 5 | <i>p,p'</i> -DDD | 49.69 | 235 | 165 |
| 5 | <i>p,p'</i> -DDT | 50.99 | 235 | 165 |

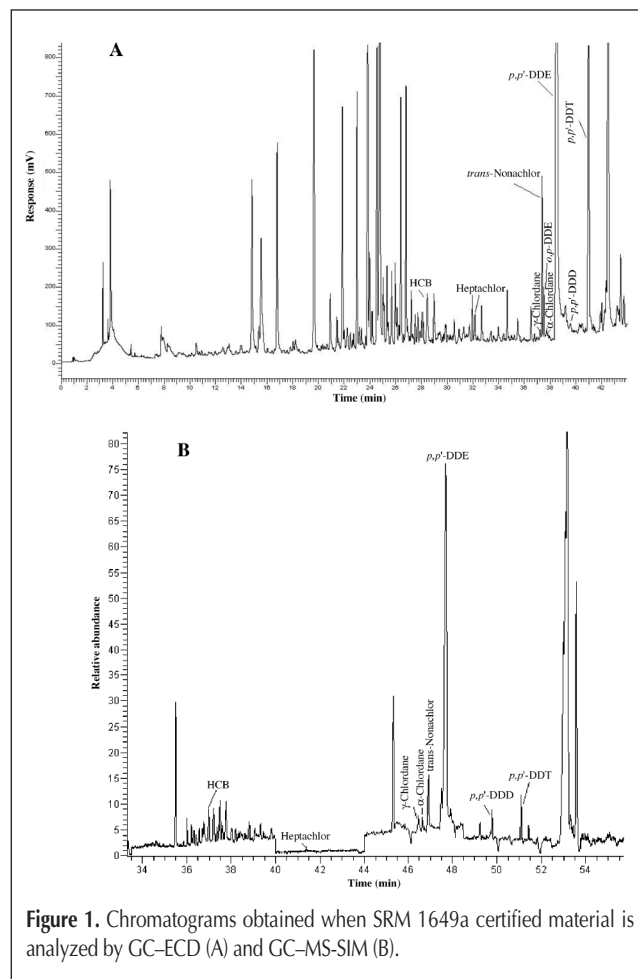


Figure 1. Chromatograms obtained when SRM 1649a certified material is analyzed by GC-ECD (A) and GC-MS-SIM (B).

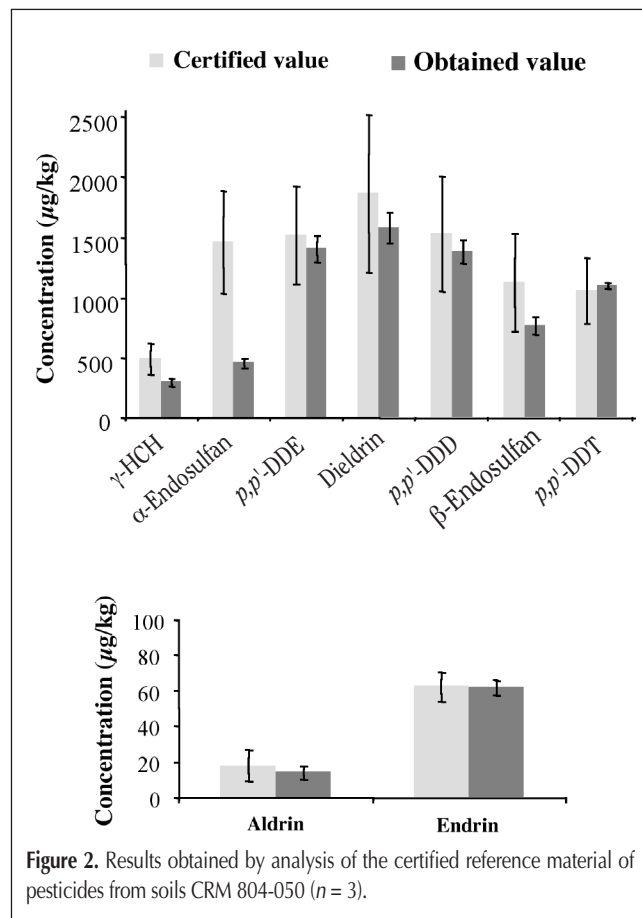


Figure 2. Results obtained by analysis of the certified reference material of pesticides from soils CRM 804-050 ($n = 3$).

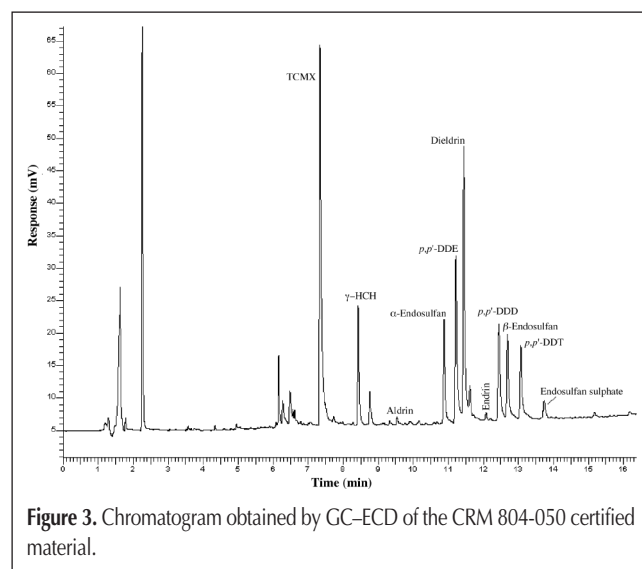


Figure 3. Chromatogram obtained by GC-ECD of the CRM 804-050 certified material.

Experimental

Analysis and quantitation

All samples and standards were analyzed by PTV-GC-ECD and PTV-GC-MS. The PTV-GC-ECD was performed with a Perkin Elmer Autosystem XL chromatograph (Norwalk, CT) equipped with ^{63}Ni ECD (350°C). The column used was a DB-35MS (Agilent Technologies, Santa Clara, DE) (30 m \times 0.32 mm \times 0.25 μm), and the oven program applied was 60°C (2 min) to 300°C (5 min) at 4.5°C/min. The injection was done in a PTV injector [60°C (0.1 min) to 300°C at 120°C/min], and 20 μL was the volume injected, as it was optimized in a previous work (12). Instrumental limits of detection were between 0.057 and 0.135 $\mu\text{g/L}$ for all the pesticides analyzed.

For the GC-MS analysis, a Thermo Finnigan gas chromatograph (Austin, TX), also equipped with PTV injector, was used. The MS detector used was a Polaris Q MS Ion trap, operating in selected ion mode (SIM) in electron impact at 70eV. The transfer line and ion source temperatures were 300°C and 250°C, respectively. The ions selected for the quantitation are in Table I. The volume injected was 8 μL , and the PTV injector program was 80°C (0.5 min) to 255°C (10 min) at 5°C/s. The chromatographic column was a DB-XLB (Agilent Technologies) (60 m \times 0.25 mm \times 0.25 μm), and the oven program was 60°C (2 min) to 300°C (5 min) at 4.5°C/min. The instrumental detection limits for the pesticides were between 1.2 and 10.7 $\mu\text{g/L}$ in the SIM mode. Samples were also injected in GC-MS in FULL (50–500 amu) mode for confirmation. In both methods, the quantitation analysis was done by internal standard calibration using TCMX as the internal standard.

Extraction procedure

The pressurized liquid extraction was carried out with an ACE 200 Accelerated Solvent Extractor (Dionex) operating in pre-heating mode (the cell was heated in the oven before solvent was introduced). The extraction and analysis procedure had been optimized for the analysis of organochlorine pesticides from soils (6) and can be summarized as follows: 1 g of sample was mixed with 0.25 g of previously washed diatomaceous earth as dispersing agent and introduced in a 5-mL PLE cell. The extraction solvent selected was a mixture of hexane-acetone (1:1, v:v) because its efficiency in the extraction of organochlorine pesticides in different matrices has been demonstrated in previous works (6,13,14). The sample was extracted at a temperature of 100°C and a pressure of 1500 psi during 5 min of static time. Activated copper was added to the extract in order to eliminate the sulphur, which causes interference in the chromatographic system. The extract was concentrated by rotary evaporator and purified with an Envi-Carb cartridge 100 m^2/g (1 g) and eluted with 10 mL of hexane-ethyl acetate (80:20, v/v). This clean up procedure gave good results when

soil samples were analyzed (6), and now the same procedure is applied to other matrices. The eluate was dried in a rotary evaporator and N_2 stream, and then it was redissolved in 1 mL of hexane with addition of the internal standard (TCMX). Finally, it was injected in both chromatographic systems (PTV-GC-ECD and PTV-GC-MS).

Table II. Accuracy Calculations for CRM804-050*

| | X_C | σ_{Lm} | X_L | S_{WL} | $ X_C - X^L $ | $2\sqrt{(\sigma_{Lm}^2 + S_{WL}^2/n)}$ | Accuracy |
|----------------------|-------|---------------|-------|----------|---------------|--|----------|
| γ -HCH | 491 | 128 | 299 | 32 | 192 | 259 | yes |
| Aldrin | 18 | 8.9 | 14.1 | 3.9 | 3.94 | 18.4 | yes |
| α -Endosulfan | 1464 | 427 | 456 | 39 | 1008 | 855 | no |
| <i>p,p'</i> -DDE | 1520 | 410 | 1406 | 110 | 114 | 830 | yes |
| Dieldrin | 1863 | 655 | 1582 | 127 | 281 | 1318 | yes |
| Endrin | 62.2 | 8.6 | 61.7 | 4.3 | 0.49 | 17.9 | yes |
| <i>p,p'</i> -DDD | 1531 | 476 | 1384 | 94 | 147 | 958 | yes |
| β -Endosulfan | 1128 | 408 | 771 | 73 | 357 | 820 | yes |
| <i>p,p'</i> -DDT | 1060 | 275 | 1101 | 26 | 41.0 | 551 | yes |

* Abbreviations are: certified value in $\mu\text{g/kg}$ (X_C); between laboratories standard deviation of the certified value (σ_{Lm}); mean value of the replicates in $\mu\text{g/kg}$ (X_L); and standard deviation of X_L (S_{WL}).

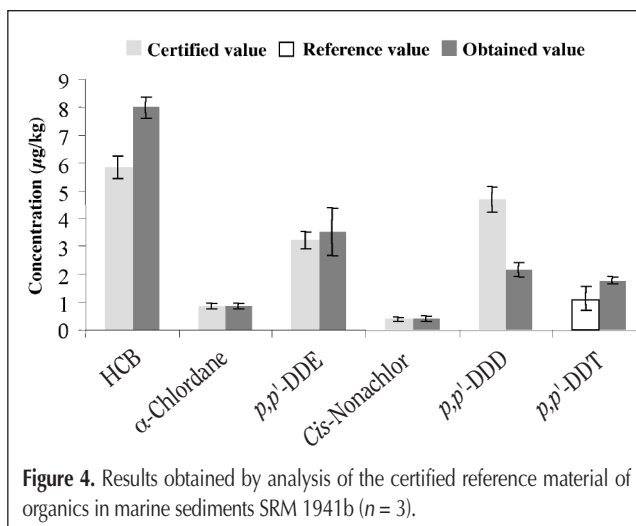


Table III. Accuracy Calculations for SRM 1941b

| | X_C | σ_{Lm} | X_L | S_{WL} | $ X_C - X^L $ | $2\sqrt{(\sigma_{Lm}^2 + S_{WL}^2/n)}$ | Accuracy |
|-----------------------|-------|---------------|-------|----------|---------------|--|----------|
| HCB | 5.8 | 0.19 | 7.9 | 0.38 | 2.14 | 0.58 | no |
| α -Chlordane | 0.85 | 0.055 | 0.84 | 0.1 | 0.01 | 0.16 | yes |
| <i>p,p'</i> -DDE | 3.2 | 0.14 | 3.5 | 0.85 | 0.29 | 1.02 | yes |
| <i>cis</i> -Nonachlor | 0.38 | 0.026 | 0.41 | 0.1 | 0.03 | 0.13 | yes |
| <i>p,p'</i> -DDD | 4.7 | 0.23 | 2.14 | 0.25 | 2.52 | 0.54 | no |
| <i>p,p'</i> -DDT | 1.12 | 0.21 | 1.78 | 0.14 | 0.66 | 0.46 | no |

* Abbreviations are: certified value in $\mu\text{g/kg}$ (X_C); between laboratories standard deviation of the certified value (σ_{Lm}); mean value of the replicates in $\mu\text{g/kg}$ (X_L); and standard deviation of X_L (S_{WL}).

Results and Discussion

To perform the experiments, all the certified reference materials, were analyzed by triplicate using the method described in the Extraction Procedure section. The extracts were injected by PTV-GC-ECD and PTV-GC-MS (SIM and FULL mode) at the condition cited in the Analysis and Quantitation section. The complexity of the matrices analyzed, considering the high number of coextracting compounds, could make the identification of the selected compounds difficult when the extracts are analyzed by GC-ECD. In this case, the use of a confirmation technique like GC-MS is very useful. Figure 1 shows, as an

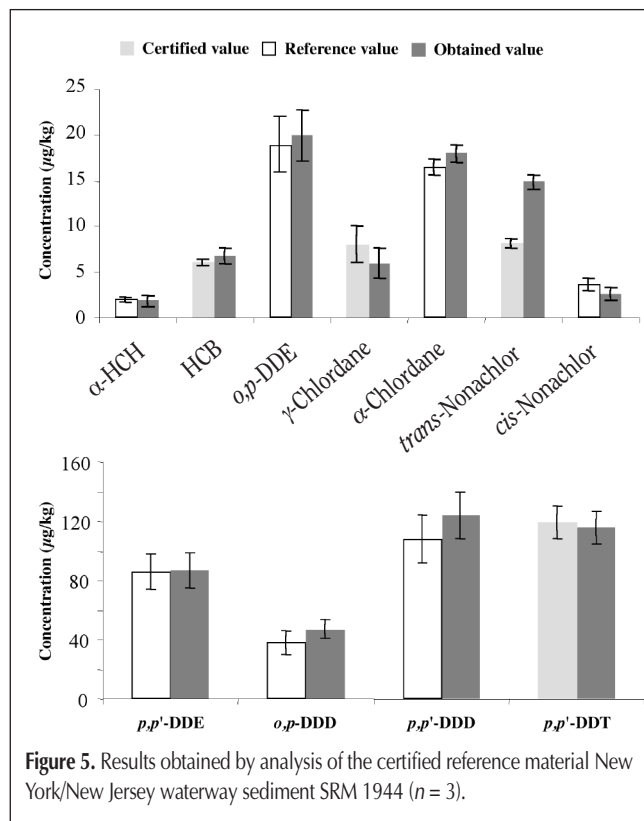


Figure 5. Results obtained by analysis of the certified reference material New York/New Jersey waterway sediment SRM 1944 ($n = 3$).

example, the chromatograms obtained for SRM1649a certified material analyzed by GC-ECD (A) and GC-MS-SIM (B).

The suitability of the method for the analysis of different matrices was evaluated extracting a certified reference material of pesticides from soil CRM804-050, two marine sediments: SRM 1941b and SRM 1944, and an urban dust SRM 1649a, which contained organochlorine pesticides in a concentration ranging between 0.6–1500 $\mu\text{g}/\text{kg}$. These certified reference materials are real-world waste samples and are affected by the same preparation problems and analytical interferences, etc, as is typical for similar matrices received in the environmental laboratories. Therefore, they are adequate to evaluate the efficiency of the proposed method.

The accuracy of the method for each certified material was evaluated according to the Canadian Certified Reference Materials Project (CCRMP)(15), which establishes that an analytical method is accepted with regard to accuracy if:

$$|X_C - X_L| \leq 2 \sqrt{(\sigma_{Lm}^2 + S_{WL}^2/n)} \quad \text{Eq. 1}$$

where X_C is the certified value, σ_{Lm} is the between-laboratories standard deviation of the certified value, X_L is the mean value of the replicates, and S_{WL} is the standard deviation of X_L .

The certified reference soil CRM804-050, collected in an agricultural region of the Western United States, contains the pesticides in a high concentration ($> 500 \mu\text{g}/\text{kg}$), except aldrin and endrin. Because of the concentration of the pesticides in this material, the analysis was done by GC-ECD using splitless injection (injector temperature 300°C, split flow 7.7 mL/min, and splitless time 1 min). The detection limits calculated at signal to noise (S/N) = 3:1 were between 3 and 10 $\mu\text{g}/\text{kg}$. As can be seen in Figure 2, the results obtained are within the confidence interval of the certified material for all the pesticides, except for an endosulfan. The anomaly low value obtained for α -endosulfan ($456 \pm 39 \mu\text{g}/\text{kg}$) was similar to that obtained when this material was extracted by other extraction procedures, such as microwave assisted extraction ($485 \pm 8.2 \mu\text{g}/\text{kg}$) and Soxhlet ($554 \pm 34 \mu\text{g}/\text{kg}$) (13). These low values could be due to the short half-life

of α -endosulfan, which is between 60 and 800 days, depending on the pH, water content, and temperature (17). The breakdown product of endosulfan is the endosulfan sulphate. Sulphate formation increases as temperatures increase (18). A peak identified as endosulfan sulphate was detected in the chromatograms (Figure 3) with a concentration of 52.3 $\mu\text{g}/\text{kg}$, verifying the degradation of α -endosulfan. The standard deviations were satisfactory with relative standard deviation lower than 10% for all the pesticides. These results are in concordance with the accuracy determination (Table II). Therefore, the developed method has demonstrated to be suitable for the extraction of several organochlorine pesticides from soils.

The next studied matrix was marine sediment. To perform this study, two marine sediment standard reference materials, SRM 1941b and SRM 1944, were analyzed using the same analytical

Table IV. Accuracy Calculations for SRM 1944.

| | X_C | σ_{Lm} | X_L | S_{WL} | $ X_C - X_L $ | $2\sqrt{(\sigma_{Lm}^2 + S_{WL}^2/n)}$ | Accuracy |
|-------------------------|-------|---------------|-------|----------|---------------|--|----------|
| α -HCH | 2 | 0.15 | 1.9 | 0.62 | 0.12 | 0.77 | yes |
| HCB | 6.0 | 0.18 | 6.7 | 0.91 | 0.71 | 1.11 | yes |
| <i>o,p</i> -DDE | 19 | 1.5 | 20 | 2.82 | 1.00 | 4.43 | yes |
| γ -Chlordane | 8 | 1 | 5.98 | 1.67 | 2.02 | 2.78 | yes |
| α -Chlordane | 16.5 | 0.42 | 18 | 0.9 | 1.50 | 1.52 | yes |
| <i>trans</i> -Nonachlor | 8.2 | 0.26 | 14.9 | 0.80 | 6.7 | 1.06 | no |
| <i>cis</i> -Nonachlor | 3.7 | 0.35 | 2.6 | 0.68 | 1.1 | 1.12 | yes |
| <i>p,p'</i> -DDE | 86 | 6 | 87 | 12 | 0.61 | 18.3 | yes |
| <i>o,p'</i> -DDD | 38 | 4 | 47 | 6.2 | 9 | 10.7 | yes |
| <i>p,p'</i> -DDD | 108 | 8 | 124 | 16 | 16 | 24.4 | yes |
| <i>p,p'</i> -DDT | 119 | 5.5 | 116 | 11 | 3.05 | 16.8 | yes |

* Abbreviations are: certified value in $\mu\text{g}/\text{kg}$ (X_C); between laboratories standard deviation of the certified value (σ_{Lm}); mean value of the replicates in $\mu\text{g}/\text{kg}$ (X_L); and standard deviation of X_L (S_{WL}).

procedure previously described. SRM 1941b “organics in marine sediments” was prepared from sediment collected in the harbor of Baltimore, Maryland, and was issued in 2002 with certified and reference concentrations assigned for 9 chlorinated pesticides. This sediment was freeze-dried and sieved (nominally $< 150 \mu\text{m}$). The values were measured with the results of the GC–MS analysis due to the presence of some interfering peaks. *trans*-Nonachlor, γ -chlordane, and *o,p*-DDE were not detected in the analysis because they were in the certified material at levels lower than the detection limits for these compounds in this matrix (about $1 \mu\text{g}/\text{kg}$). To detect these pesticides at the very low levels certified it is necessary to analyze a larger amount of sample similar to the amount analyzed in the certification of the material (10 g for GC–ECD analysis and 100 g for GC–MS). However, as it can be seen in Figure 4, good concordance between the obtained results and the certified concentration were observed for α -chlordane, *p,p'*-DDE, and *cis*-nonachlor by extraction of just 1 g of sample. The same results were obtained when accuracy was determined (Table III). For *p,p'*-DDD and *p,p'*-DDT, no concordance was obtained, possibly because of the low concentration of these compounds in the SRM 1941b sediment. This is corroborated by the good results obtained for these compounds in the other reference materials.

SRM 1944 “New York/New Jersey waterway sediment” is

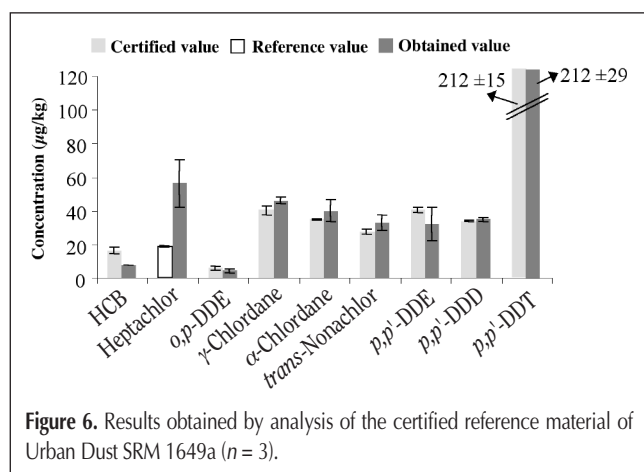


Figure 6. Results obtained by analysis of the certified reference material of Urban Dust SRM 1649a ($n = 3$).

highly contaminated marine sediment that was prepared from sediment collected from six different sites in the New York and New Jersey bays. During preparation of the SRM the sediment was sieved, resulting in a particle size that is nominally between $61\text{--}250 \mu\text{m}$. The levels of pesticides are 5–10 times higher in this SRM material than in SRM 1941b. As Figure 5 shows, the values obtained for most of the pesticides have good concordance with the certified and reference values. Only for *trans*-nonachlor was the value obtained anomaly high with unsatisfactory accuracy (Table IV). The good results obtained for this compound in the particulate matter material (SRM 1649a) could indicate the presence of a matrix interference in the SRM 1944. The standard deviation of the results was also satisfactory and similar to the deviation of the certified material. The detection limits obtained for the pesticides in this matrix, calculated at $S/N = 3:1$, were between 0.03 and $4 \mu\text{g}/\text{kg}$ (PTV–GC–MS–SIM analysis).

Finally, a very different matrix SRM 1649a “urban dust” was also analyzed. This reference material was prepared from atmospheric particulate matter collected in Washington DC over a period in excess of 12 months and therefore represents a time-integrated sample. This sample should generally typify atmospheric particulate matter obtained from an urban area.

When the urban dust + diatomaceous earth mixture is introduced on the 5-mL PLE cell, this cell is almost completely filled (whereas with the other materials evaluated the cell is filled c.a. 1/3). This is due to the low density of this material. However, no differences were observed in the final volume of the PLE extract.

Many interfering compounds were observed in the chromatogram obtained when this urban dust PLE extract was injected, even though the results obtained for this material were quite good (Figure 6). A high value for heptachlor was obtained. This compound has a low signal when analyzed by GC–MS, making it more affected by the background noise caused by the matrix. Furthermore, the value for this compound in the reference material is a reference value, not a certified value. The results in Table V show good accuracy for all the pesticides, except HCB, heptachlor, and γ -chlordane, although for this compound the recovery obtained was 113%. The detection limits for the pesticides in this material were between 0.5 and $2.8 \mu\text{g}/\text{kg}$ (PTV–GC–MS–SIM analysis).

Table V. Accuracy Calculations for SRM 1649a. X_C : Certified Value ($\mu\text{g}/\text{kg}$); σ_{LM} : Between Laboratories Standard Deviation of the Certified Value; X_L : Mean Value of the Replicates ($\mu\text{g}/\text{kg}$); S_{WL} : Standard Deviation of X_L .

| | X_C | σ_{LM} | X_L | S_{WL} | $ X_C - X_L $ | $\frac{2\sqrt{(\sigma_{LM}^2 + S_{WL}^2/n)}}{X_C}$ | Accuracy |
|-------------------------|-------|---------------|-------|----------|---------------|--|----------|
| HCB | 16.3 | 0.9 | 7.5 | 0.11 | 8.78 | 1.80 | × |
| Heptachlor | 18.9 | 0.25 | 56 | 14.0 | 37.5 | 16.2 | × |
| <i>o,p</i> -DDE | 5.8 | 0.42 | 4.3 | 1.24 | 1.50 | 1.95 | ✓ |
| γ -Chlordane | 40 | 1.4 | 46 | 1.81 | 5.40 | 3.49 | × |
| α -Chlordane | 35 | 0.21 | 40 | 6.61 | 5.31 | 7.65 | ✓ |
| <i>trans</i> -Nonachlor | 28 | 0.8 | 33 | 4.5 | 5.00 | 6.56 | ✓ |
| <i>p,p'</i> -DDE | 40 | 0.85 | 32 | 9.7 | 8.39 | 11.33 | ✓ |
| <i>p,p'</i> -DDD | 34 | 0.24 | 35 | 1.1 | 1.20 | 1.36 | ✓ |
| <i>p,p'</i> -DDT | 212 | 7.5 | 212 | 28.7 | 0 | 36.43 | ✓ |

Conclusion

The proposed and evaluated analytical procedure of pressurized liquid extraction and carbon cleanup has demonstrated its suitability for the analysis of organochlorine pesticides in several solid matrices in a broad range of concentrations. The different nature of the materials assayed and their complexity could cause some problems in the correct chromatographic identification and integration; therefore, GC–MS is recommended as a confirmation technique. The detection limits obtained were different for each matrix. Despite these difficulties, good concor-

dance between the concentration obtained and the certified values was achieved for the majority of the pesticides.

Acknowledgment

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