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# Selective extraction of hydrocarbons, phosphonates and phosphonic acids from soils by successive supercritical fluid and pressurized liquid extractions

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## Abstract

Hydrocarbons, dialkyl alkylphosphonates and alkyl alkylphosphonic acids are selectively extracted from spiked soils by successive implementation of supercritical carbon dioxide, supercritical methanol–modified carbon dioxide and pressurized water. More than 95% of hydrocarbons are extracted during the first step (pure supercritical carbon dioxide extraction) whereas no organophosphorus compound is evidenced in this first extract. A quantitative extraction of phosphonates is achieved during the second step (methanol–modified supercritical carbon dioxide extraction). Polar phosphonic acids are extracted during a third step (pressurized water extraction) and analyzed by gas chromatography under methylated derivatives (diazomethane derivatization). Global recoveries for these compounds are close to 80%, a loss of about 20% occurring during the derivatization process (co-evaporation with solvent). The developed selective extraction method was successfully applied to a soil sample during an international collaborative exercise. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** Pressurized liquid extraction; Extraction methods; Soil analysis; Hydrocarbons; Phosphonates; Phosphonic acids

## 1. Introduction

The Convention on Chemical Weapons which entered into force on 29 April 1997 bans development, production, stockpiling and use of chemical warfare agents (CWAs). Restricted compounds are classified into three schedules which cover CWAs, their precursors and their degradation products [1]. The major part of classified chemicals are organophosphorus compounds. Consequently, the verification of the fulfillment of the treaty requires the

development of dedicated methods allowing the detection of these compounds in samples of various origin (suspected production sites, storage sites, environment in case of alleged use).

Collected samples are often highly contaminated with hydrocarbons (diesel oil, gasoline, oil, etc.). Therefore, traditional non selective ultrasonic extraction [2] produces extracts containing a high level of these compounds which can greatly complicate identification of restricted compounds [3–5].

The aim of this work is to develop a three-step extraction procedure in order to selectively extract hydrocarbons (sample clean-up), phosphonates

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(classified compounds which can be used as precursors of nerve agents) and phosphonic acids (classified compounds stemming from fast hydrolysis of nerve agents). Restricted organophosphorus compounds contained in these fractions will be easily identified by spectrometric methods like mass spectrometry or nuclear magnetic resonance for example. Pure supercritical carbon dioxide is employed during the first step. Indeed, as previously described, CO<sub>2</sub> has proven to be efficient in removing total petroleum hydrocarbons from soils [3–5]. The second step, performed with supercritical methanol–carbon dioxide mixture, is assumed to quantitatively extract moderately polar phosphonates [6,7]. Finally, pressurized water, implemented during the third step, is assumed to lead to a quantitative extraction of polar phosphonic acids.

Optimization of selective extraction procedure is reported. Then, this procedure is applied to a soil

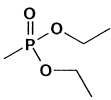
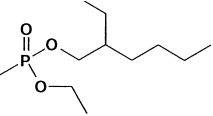
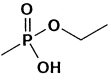
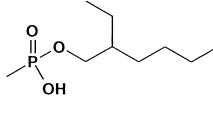
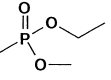
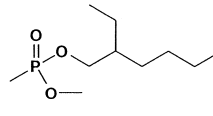
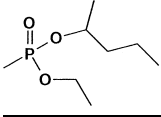
sample that was analyzed during the fifth international collaborative exercise conducted by the Technical Secretariat for the Organization for the Prohibition of Chemical Weapons based in The Hague. During this round robin test, it was asked to the participating laboratories applying for designation for verifying the application of the Chemical Weapons Convention to identify scheduled compounds possibly contained in various matrices at a level ranging from 1 to 10 ppm.

## 2. Experimental

### 2.1. Chemicals

Organophosphorus compounds involved in this study are listed in Table 1. They were synthesized in the laboratory at the Centre d'Etudes du Bouchet, a

Table 1  
Compounds synthesized for this study

| Structure   | Name                                    | Purity ( <sup>31</sup> P-NMR) | No.                   |
|---|---|-------------------------------|-----------------------|
|   | Diethyl methylphosphonate               | >97%                          | 1                     |
|  | Ethyl (2-ethylhexyl) methylphosphonate  | >99%                          | 2                     |
|  | Ethyl methylphosphonic acid             | >98%                          | 3                     |
|  | (2-Ethylhexyl) methylphosphonic acid    | >98%                          | 4                     |
|  | Ethyl methyl methylphosphonate          | >92%                          | 5                     |
|  | (2-Ethylhexyl) methylphosphonate        | >99%                          | 6                     |
|  | Ethyl (1-methylbutyl) methylphosphonate | >97%                          | 7 (internal standard) |

French defense research establishment (Le Bouchet, France). A commercial diesel oil was provided by a local supplier.

## 2.2. Solvents

Ethyl acetate, methanol and hexane (Pestnorm grade) were provided by Prolabo (Nogent sur Marne, France). Deionized water was freshly prepared by the alpha-Q water purification system (Millipore, Bedford, MA, USA).

## 2.3. Soil preparation and spiking

Two soils were employed in this study. The first one (called “local soil”) was collected close to our laboratory whereas the second one comes from French Guyana. The characteristics of the two soils, determined by the French National Institute of Agronomic Research (INRA, Olivet, France) are given in Table 2. Before being spiking, the two soils were allowed to dry at 40°C for a week and sieved at 2 mm.

Soils spiking was performed as follows. A 5-ml volume of a 25 mg/ml diesel oil solution in hexane was added to 100 g of soil contained in a 250-ml Erlenmeyer flask (hydrocarbons pollution level: 1250 µg/g). The resulting mixture was agitated during 2 min with a vortex mixer. After a 1-h waiting period (hexane evaporation), the soil was successively spiked with compounds **1** and **2** (respective addition of 1 ml of a 1 mg/ml solution in ethyl acetate). The soil was then vortex-mixed during 2 min. One hour later, compounds **3** and **4** were introduced to the soil by respective addition of 1 ml of a 1 mg/ml compound **3** solution in methanol and 1 ml of a 1 mg/ml compound **4** solution in methanol (compounds **1** to **4** pollution level: 10 µg/g). The resulting mixture was agitated during 2 min with a vortex mixer. After solvent evaporation (24 h at room temperature), the screw-capped vessel was

closed and the soil was stored at room temperature until extraction procedure occurs.

## 2.4. Supercritical fluid extraction (SFE)

All the SFE experiments were carried out in triplicate on an HP7680A Supercritical Fluid Extractor (Hewlett-Packard France, Les Ulis, France) in a two-step manner. First, sample was accurately weighed (between 9 and 10 g per cell) into the 7-ml extractor thimble and pure supercritical carbon dioxide extraction was implemented (hydrocarbons selective extraction). Immediately after this run, 700 µl of methanol (i.e., 10% of the extraction cell volume) was added to the top of the cell and a second extraction was performed allowing phosphonates selective extraction (operating conditions of the two extractions are shown in Table 3).

The extractor trapping system was modified as previously described [8] in order to check the trap efficiency when SFE is performed with methanol-modified carbon dioxide. During extraction of a spiked soil (second step: addition of 700 µl of MeOH), expanded supercritical fluid percolates through two successive traps filled with a high specific area (1100 m<sup>2</sup>/g) styrene-divinylbenzene copolymer (Isolut ENV+, International Sorbent Technology, provided by Touzart et Matignon). It was shown that all extracted compounds are collected in the trap. Consequently, in this study, only the first trap will be used. Indeed, as previously described [9], the solid trapping remains efficient with a moderate content of modifier (up to 10%) added to supercritical carbon dioxide provided that a high specific area polymeric phase is used instead of a classical octadecyl silica.

Moreover, it was also shown that 1.8 ml is the lowest volume of ethyl acetate which allows a total elution of solutes from the trap.

A 75-µl volume of an internal standard solution (compound **7** in ethyl acetate at 1058 µg/ml) was added to the two extracts.

Table 2  
Characteristics of the two investigated soils (INRA analysis)

| Soil type   | Sand (%) | Silt (%) | Clay (%) | pH  | Organic matter (%) |
|-------------|----------|----------|----------|-----|--------------------|
| Local soil  | 28.9     | 51.1     | 20.0     | 6.9 | 2.6                |
| Guyana soil | 52.9     | 29.2     | 17.9     | 4.8 | 7.7                |

Table 3  
SFE operating conditions

|   | First step                              | Second step   |
|---|---|---------------|
| Temperature (°C)                                    | 60                                      | 80            |
| Pressure (bar)                                      | 129                                     | 202           |
| Density (g/ml)                                      | 0.5                                     | 0.6           |
| Modifier  | No                                      | 700 µl (MeOH) |
| Static period (min)                                 | 0.2                                     | 10–40         |
| Dynamic period (min)                                |   | 20            |
| Flow-rate, measured at the liquid state (ml/min)    |   | 1             |
| Trapping material                                   | ENV+ (styrene–divinylbenzene copolymer) |               |
| Elution solvent                                     | Ethyl acetate                           |               |
| Elution volume (ml)                                 | 1.8                                     |               |
| Elution flow-rate (ml/min)                          | 1                                       |               |
| Trap temperature during extraction and elution (°C) | 25                                      |               |
| Nozzle temperature (°C)                             | 45                                      |               |

### 2.5. Pressurized liquid extraction (PLE)

Extractions were performed with a Dionex ASE 200 system (Jouy en Josas, France). After the two supercritical fluid extractions, the sample was transferred to a PLE cell (11 ml). Void volume was filled with glass beads. A cellulose filter (diameter: 19.1 mm, type D28) supplied by Dionex was routinely disposed at the exit of the cell to prevent clogging of the metal frit. Extraction starts with a filling step: water is pumped through the cell. When the cell is full and the collection vial contains about 1 ml of water, the static valve is closed and the pump is automatically stopped. The next step (preheating) provides thermal sample homogeneity. It takes 5 min (respectively, 7 min) for an extraction temperature of 80°C (respectively, 150°C). During this step, the static valve is sometimes opened to maintained the pre-set pressure (100 bar). After that, a static extraction is realized (10 min). At the end of this static period, the static valve is opened and 3.5 ml of fresh water are pumped through the cell (flush). This cycle (static period+flush) is repeated three times. Finally, the extraction cell is purged with nitrogen (12 bar) during 90 s to assure a complete water transfer to the collection vial.

### 2.6. Gas chromatography

Extracts were analyzed on a Varian 3400 gas chromatograph (Varian, Les Ulis, France) equipped with a flame ionization detection (FID) system, a

flame photometric detection (FPD, phosphorous mode) system, a split/splitless injector (Varian 1077) and an autosampler (Varian 8200). A Restek Rtx-5MS (5% biphenyl, 95% methylpolysiloxane) with an integrated guard column was used (30 m×0.32 mm I.D., film thickness 0.25 µm). The autosampler injections (1 µl) were performed in the splitless mode for 0.75 min. The oven temperature was held at 50°C for 1 min then ramped to 260°C at 10°C/min. The injector and detectors temperatures were set at 250°C and 260°C, respectively. Linear correlation coefficients ( $r^2$ ) for all calibration curves were always greater than 0.995 (only FPD-P was used for quantification, results being based on triplicate injections).

A diazomethane methylation, implemented on water extracts (which are assumed to contain polar compounds **3** and **4**), leads to compounds **5** and **6** which could be analyzed by gas chromatography (Fig. 1). A diazomethane solution in ether was prepared weekly by reaction of potassium hydroxide with *N*-nitroso *N*-methylurea [10].

## 3. Results and discussion

### 3.1. $CH_2N_2$ derivatization

Diazomethane derivatization efficiency was checked as followed. A pressurized water extract (150°C) of a blank Guyana soil sample was spiked with 100 µg of each compounds **3** and **4** (simulation

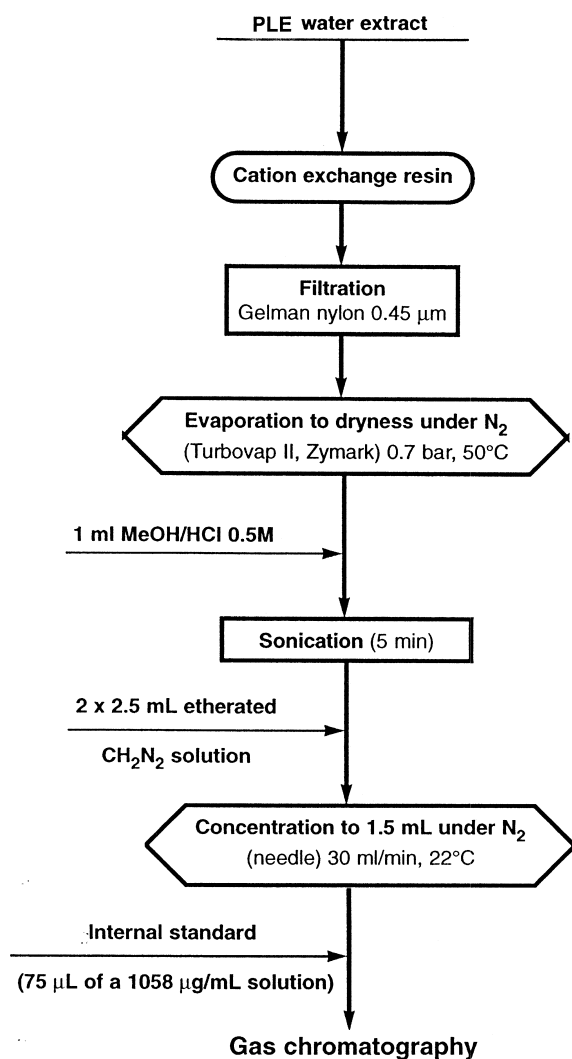


Fig. 1. Diazomethane methylation of pressurized water extracts.

of a quantitative extraction of 10 g of spiked soil). The derivatization process (Fig. 1), implemented on this aqueous solution, leads to compounds **5** and **6** which are quantified by gas chromatography (FPD-P). This procedure allows one to determine a derivatization step yield of 82.9% (RSD=5.8%) and 81.6% (RSD=7.0%) for compounds **3** and **4**, respectively.

Taking into account the derivatization step losses (due to the co-evaporation of target compounds with solvent molecules), the global recoveries of phosphonic acids, measured during the complete process

(pressurized water extraction and  $\text{CH}_2\text{N}_2$  derivatization), cannot be higher than 80%.

Similar results were obtained with a spiked water extract (150°C, 100 bar) of local soil.

### 3.2. Guyana soil extraction

A three-step extraction was performed on spiked Guyana soil. As expected, the first step, performed with pure supercritical carbon dioxide, allows one to extract most of the hydrocarbons (95% of spiked hydrocarbons) whereas organophosphorus compounds remain in the sample (no compounds were evidenced in FPD-P chromatogram of this first extract). As a second step, a methanol-modified supercritical carbon dioxide extraction was implemented to remove alkyl alkylphosphonates (compounds **1** and **2**). The influence of the static period duration on the recoveries of compounds **1** and **2** is represented in Fig. 2. From now on, a static period of 20 min, which allows one to extract 91.0% (RSD=2.1%) and 86.0% (RSD=1.9%) of compounds **1** and **2**, respectively will be implemented (compromise between sample treatment duration and target compounds recoveries).

Finally, a water pressurized extraction is implemented to extract polar phosphonic acids. It appears that temperature has a tremendous influence

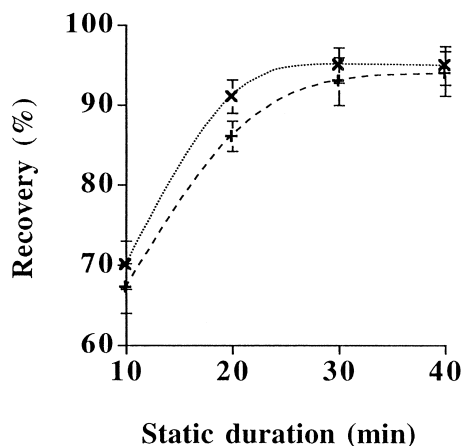


Fig. 2. Influence of the static duration on the recoveries of compounds **1** (×) and **2** (+) during the methanol-modified supercritical carbon dioxide extraction. Vertical bars represent the relative standard deviations. Extraction conditions: see Table 3.

Table 4

Temperature influence on recovery of compounds **3** and **4** (analyzed under methylated form) during the third step (water pressurized extraction–CH<sub>2</sub>N<sub>2</sub> derivatization)

| Temperature (°C) | % Recovery (% RSD based on triplicate extractions)    |   |
|------------------|---|---|
|                  | Compound <b>3</b><br>(analyzed under methylated form) | Compound <b>4</b><br>(analyzed under methylated form) |
| 80               | 50.2 (5.5)  | 7.7 (10.2)  |
| 150              | 77.7 (6.4)  | 76.6 (9.9)  |

on the recovery of this step (Table 4). A temperature of 150°C leads to a quantitative extraction step of compounds **3** and **4** from Guyana soil (total recoveries are close to 80% due to the losses occurring during the CH<sub>2</sub>N<sub>2</sub> derivatization). Polar compounds **3** and **4** develop strong interactions with soil active sites. An increase of thermal energy allows a more efficient disruption of these bonds and therefore an increase of target compounds extraction recoveries. Influence of extraction pressure was not investigated because it is now well-known that this factor has no influence on the extraction efficiency of compounds contained in dry soils. No thermal degradation of

compounds **3** and **4** (which would lead to methylphosphonic acid analyzed under dimethylated derivative) is observed.

FPD-P and FID gas chromatograms of Guyana soil successive extracts are respectively represented in Figs. 3 and 4. As expected, profile of the FID gas chromatogram of the first extract (Fig. 4a) is similar to the one obtained from injection of diesel oil used to spiked soil samples (Fig. 4d). Residual hydrocarbons are extracted during the second step (supercritical methanol–modified carbon dioxide) whereas no hydrocarbon is present in the third extract (pressurized water extraction). Compound **7**, which pre-

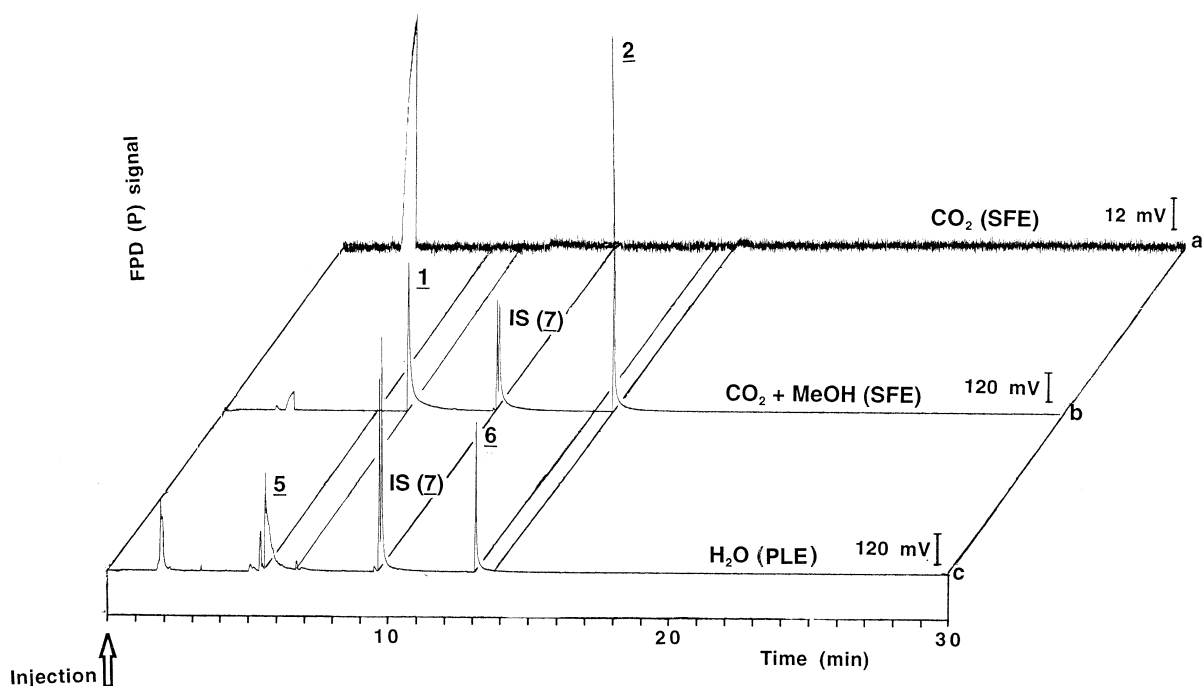


Fig. 3. FPD-P gas chromatograms of successive Guyana soil extracts obtained by pure supercritical carbon dioxide (a), methanol–modified supercritical carbon dioxide (b) and pressurized water (c). Extraction conditions: static duration of second SFE step: 20 min, water temperature during PLE (third step): 150°C, for other parameters, see Table 3.

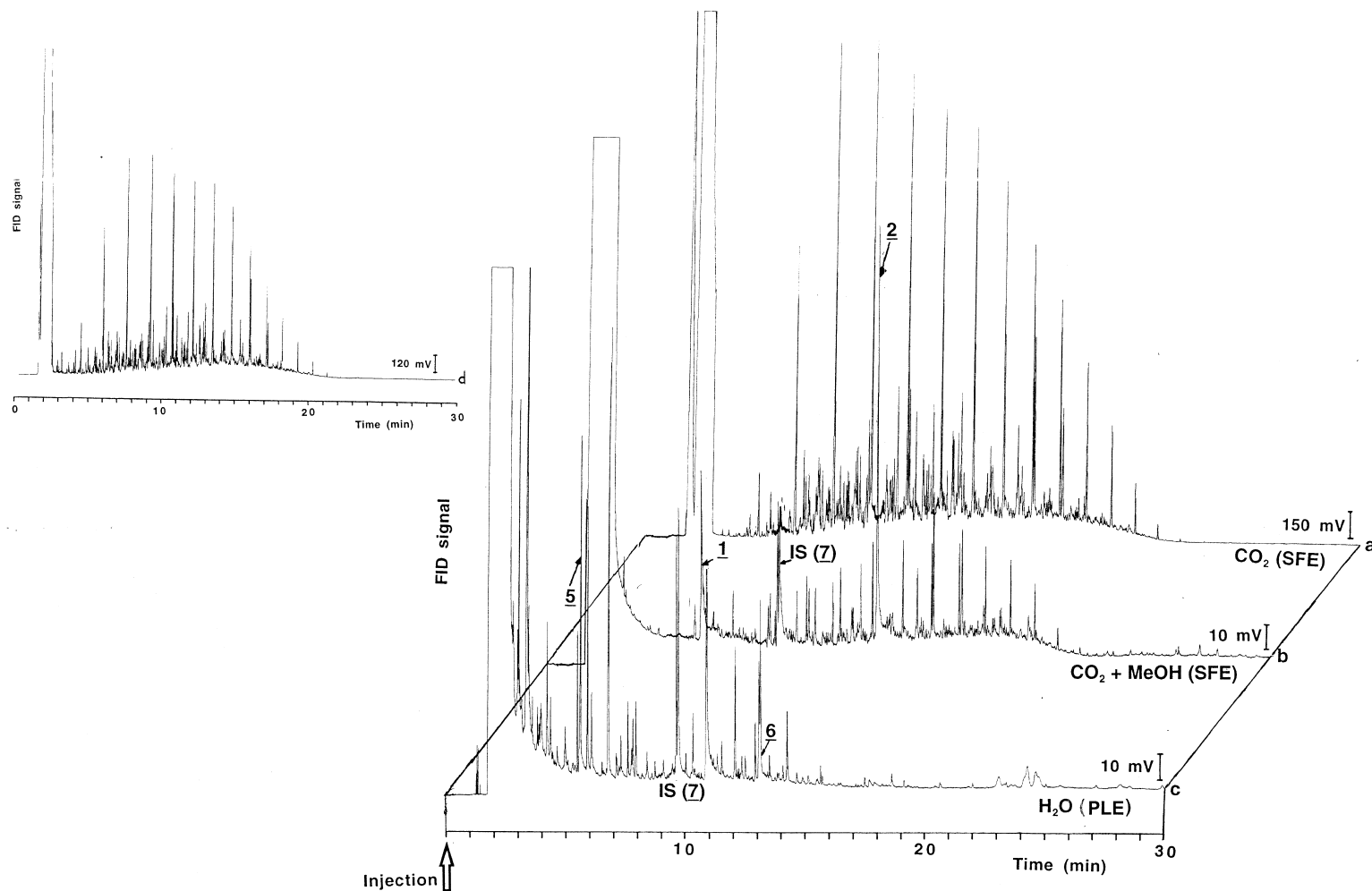


Fig. 4. FID gas chromatograms of successive Guyana soil extracts obtained by pure supercritical carbon dioxide (a), methanol–modified supercritical carbon dioxide (b) and pressurized water (c). FID gas chromatogram of diesel oil used to spiked soil sample is represented in (d). Extraction conditions: static duration of second SFE step: 20 min, water temperature during PLE (third step): 150°C, for other parameters, see Table 3.

Table 5

Recoveries of successive extractions implemented on local soil [step 1: pure supercritical carbon dioxide extraction; step 2: methanol–modified supercritical carbon dioxide extraction (static period 20 min); step 3: pressurized water extraction–diazomethane derivatization (temperature: 150°C)]

| Compound             | % Recovery (% RSD based on triplicate extractions) |             |            |
|----------------------|--|-------------|------------|
|                      | Step 1   | Step 2      | Step 3     |
| Hydrocarbons         | 95.5 (3.1)   | 4.5 (4.0)   | 0          |
| <b>1</b>             | ND <sup>b</sup>                                    | 100.6 (2.6) | ND         |
| <b>2</b>             | ND   | 98.7 (3.2)  | ND         |
| <b>3<sup>a</sup></b> | X <sup>b</sup>                                     | X           | 68.3 (6.2) |
| <b>4<sup>a</sup></b> | X  | X           | 71.2 (5.2) |

<sup>a</sup> Analyzed under methylated form (CH<sub>2</sub>N<sub>2</sub> derivatization).

<sup>b</sup> ND=Not detected, X=GC analysis, which requires a derivatization step, was not performed. For extraction conditions see Table 3.

sents two asymmetric centers, elutes as two peaks, each corresponding to a diastereoisomer couple. Compounds **2** and **6** give a single peak whereas they present two asymmetric centers.

### 3.3. Local soil extraction

Optimized conditions for Guyana soil (static duration of 20 min during the second SFE step, temperature of 150°C during pressurized water extraction) were applied to spiked local soil. Similarly to Guyana soil samples, implementation of pure supercritical carbon dioxide leads to a quasi-quantitative extraction of hydrocarbons whereas compounds **1** and **2** (dialkyl alkylphosphonates) are completely extracted with methanol–modified supercritical carbon dioxide (Table 5). Finally, alkyl alkylphosphonic acids (compounds **3** and **4**) are extracted by pressurized water and analyzed (global recoveries are close to 70%, corresponding to a quantitative extraction step).

### 3.4. Round robin sample

The selective extraction procedure developed in previous sections was applied to a soil sample

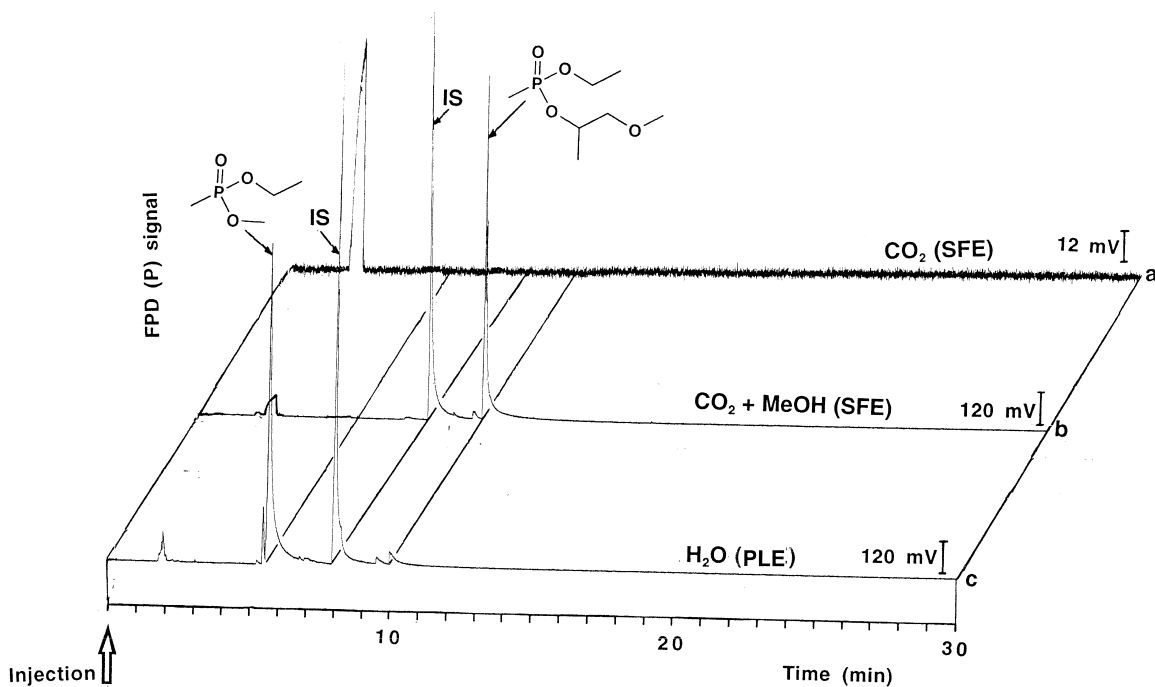


Fig. 5. FPD-P gas chromatograms of successive round robin sample (L98SO1) extracts obtained by pure supercritical carbon dioxide (a), methanol–modified supercritical carbon dioxide (b) and pressurized water (c). Extraction conditions: static duration of second SFE step: 20 min, water temperature during PLE (third step): 80°C, for other parameters, see Table 3.



(L98SO1) analyzed during an international collaborative exercise. The aim of this latter (proficiency test), conducted by the Technical Secretariat for the Organization for the Prohibition of Chemical Weapons, was to test the ability of participating laboratories (20 laboratories from 17 countries) to undoubtedly identify classified compounds contained in various matrices at a ppm level.

FPD-P and FID gas chromatograms of L98SO1 successive extracts (after addition of internal standard) are respectively represented in Figs. 5 and 6. Methanol-modified supercritical CO<sub>2</sub> and pres-

surized water extracts are particularly clean because of the efficiency of pure supercritical carbon dioxide extraction to quantitatively remove hydrocarbons.

In a first step, ethyl [(1-methyl, 2-methoxy)ethyl] methylphosphonate (compound **8**) and ethyl methylphosphonic acid (compound **3**) were identified by GC–MS in the second (MeOH-modified supercritical CO<sub>2</sub>) and in the third (pressurized water–CH<sub>2</sub>N<sub>2</sub> derivatization) extracts, respectively. After that, internal standard (ethyl propyl methylphosphonate, <sup>31</sup>P-NMR purity higher than 99%) was added to the extracts. This compound was selected because com-

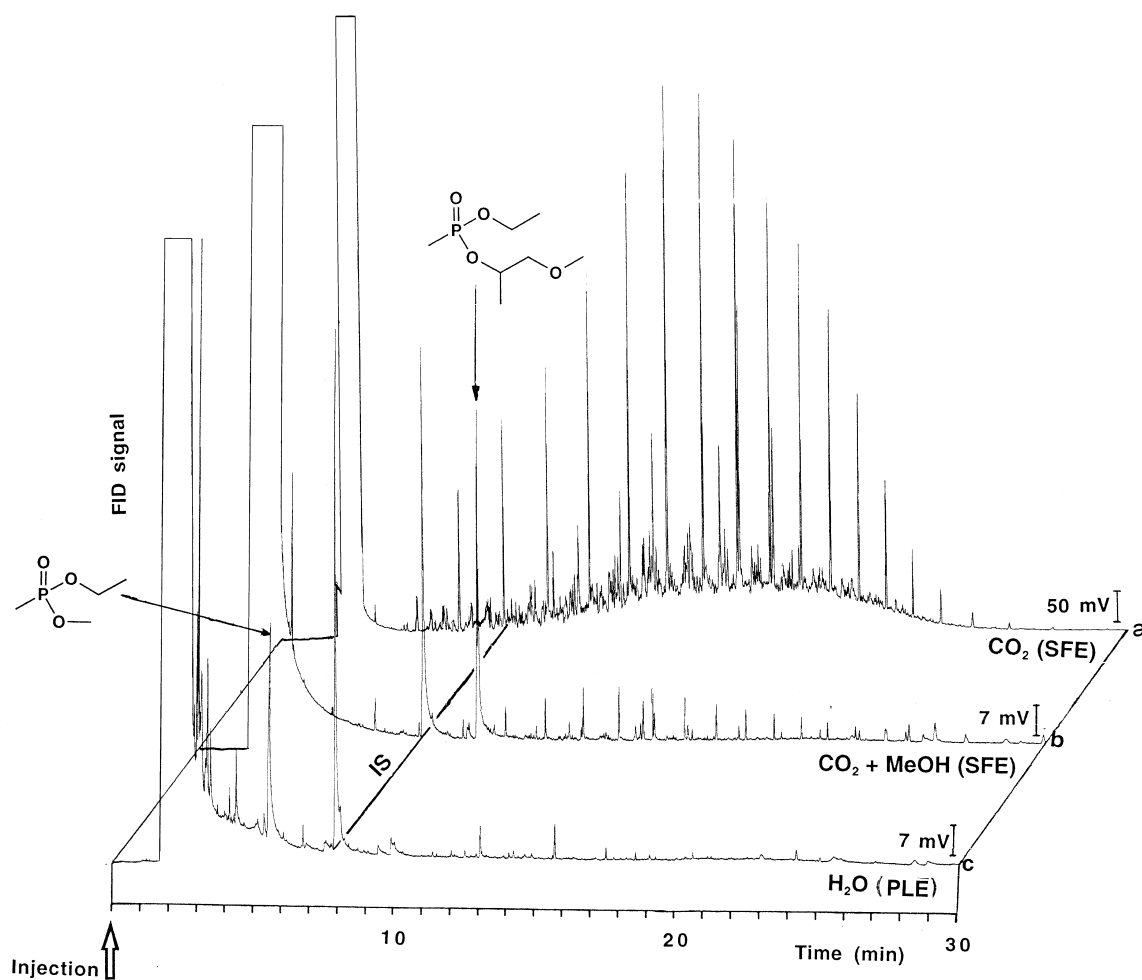
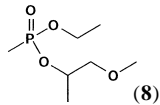
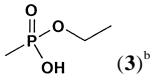


Fig. 6. FID gas chromatograms of successive round robin sample (L98SO1) extracts obtained by pure supercritical carbon dioxide (a), methanol-modified supercritical carbon dioxide (b) and pressurized water (c). Extraction conditions: static duration of second SFE step: 20 min, water temperature during PLE (third step): 80°C, for other parameters, see Table 3.

Table 6

Recoveries of successive extractions implemented on round robin sample (L98SO1) [step 1: pure supercritical carbon dioxide extraction; step 2: methanol–modified supercritical carbon dioxide extraction (static period 20 min); step 3: pressurized water extraction–diazomethane derivatization (temperature: 80°C)]

| Compound   | Recovery (%) <sup>a</sup> |        |        |
|--|---------------------------|--------|--------|
|  | Step 1                    | Step 2 | Step 3 |
| Hydrocarbons   | 99.5                      | 0.5    | 0      |
|  (8)              | ND <sup>c</sup>           | 91.2   | 1.0    |
|  (3) <sup>b</sup> | X <sup>c</sup>            | X      | 83.1   |

<sup>a</sup> Only one experiment was implemented because of lack of sample.

<sup>b</sup> Analyzed under methylated form (CH<sub>2</sub>N<sub>2</sub> derivatization).

<sup>c</sup> ND=Not detected, X=GC analysis, which requires a derivatization step, was not performed. For extraction conditions see Table 3.

compound **7** (used as internal standard in previous sections) co-elutes with compound **8**.

At the end of the exercise, it was disclosed that sample is spiked with 10 µg/g of the two previous identified compounds. It was then possible to calculate recoveries of the different extraction steps (Table 6) and to demonstrate that the two spiked compounds were quantitatively extracted (as previously described, a quantitative extraction of phosphonic acids leads to a global recovery of about 80% due to the losses occurring during the derivatization step). Note that a temperature of 80°C was employed during pressurized water extraction in order to prevent thermal degradation of others compounds possibly contained in the sample.

#### 4. Conclusion

The successive implementation of supercritical

carbon dioxide, methanol–modified carbon dioxide and pressurized water leads to a selective extraction of hydrocarbons, dialkyl alkylphosphonates and alkyl alkylphosphonic acids contained in different soils.

The developed method is particularly useful in verification of the fulfillment of the Chemical Weapons Convention. Indeed, this method allows to determine easily organophosphorus compounds, which constitute a major part of scheduled chemicals, in solid samples highly contaminated with hydrocarbons (a majority of real samples).

It was demonstrated that a temperature as high as 150°C can be used during water extraction without degradation of alkyl methylphosphonic acids.

Finally, the developed method was successfully applied to a soil sample during an international collaborative exercise.

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