

Trace level detection and identification of chemicals related to the chemical weapons convention from complex organic samples[☆]

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Abstract

A solid-phase extraction (SPE) method involving selective usage of solvents has been developed for trace level identification of chemical warfare agents (CWAs) present in a complex organic background. The total ion chromatograms obtained from direct gas chromatography–electron ionization mass spectrometric analysis of samples spiked with CWAs in the presence of diesel are very complex and dominated by hydrocarbon peaks and the same after treatment with SPE show distinct peaks corresponding to spiked chemicals. The recovery of samples from SPE is found to be 70–85% at the 10 ppm level.

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1. Introduction

Chemical warfare agents (CWAs) are potent toxic chemicals used for mass destruction. The Organization for the Prohibition of Chemical Weapons (OPCW), seated in The Netherlands regulates the production, stockpiling and usage of these chemicals produced in various countries. These chemicals are classified into three groups, namely schedules 1, 2 and 3, according to the chemical weapons convention (CWC). The OPCW conducts the proficiency tests to designate laboratories worldwide for the off-site analysis of the chemicals related to CWC [1,2]. In these tests, complex matrices such as soil, water, organic liquids, etc. spiked with parts per million levels of CWC-related chemicals along with background/masking compounds like diesel, poly(ethylene glycol) (PEG), etc. are given to participating laboratories for their detection and identification within 15 calendar days. The corresponding blank samples with or without background material are also given for comparison purpose.

Several laboratories are working in this area to develop extraction and detection methods for trace level identification of CWAs. Classical extraction methods extract the spiked chemicals together with background material. It is possible to detect phosphorous, sulfur and nitrogen containing CWAs in the presence of complex background by using GC coupled with element-specific detection such as nitrogen–phosphorous detection (NPD) and flame photometric detection (FPD) [3–7]. The use of NPD and FPD give tentative identification of compounds containing nitrogen, phosphorus and sulfur atoms, but unambiguous identification is possible only with GC–electron ionization (EI) MS analysis. In some cases, liquid chromatography coupled with electrospray ionization mass spectrometry (LC-ESI-MS) is also proven to be an efficient technique for providing structural information [8,9]. The conventional GC–EI-MS and GC–chemical ionization (CI) MS methods are routinely used to detect and identify the CWAs. The compounds can be identified from the EI mass spectrum only if the spectrum is clean and comparable to that of reference spectrum from library or authentic sample. But the GC–MS chromatograms obtained from OPCW test samples after extracting by classical methods, especially the sample in organic matrices, are very complex. The hydrocarbon background masks the compounds and the EI mass spectra contain interfering peaks due to co-eluting background

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peaks that make it difficult to identify the peak of interest. In such cases, sample extraction methods are very crucial for selective removal of background to detect CWAs in the presence of complex background material. Solid-phase extraction (SPE) and solid-phase microextraction (SPME) have been extensively used as the sample preparation techniques for the analysis of toxic chemicals present in aqueous samples [10–26]. However, there are no reports of using SPE for the analysis of CWAs present in complex organic backgrounds. In the present study, we report a simple and versatile analytical technique based on SPE for the detection and identification of CWAs in complex hydrocarbon masked organic liquid samples. The method has also been successfully adopted during the 14th OPCW proficiency test and the spiked chemicals were identified unambiguously because of high enrichment of sample during the applied SPE method.

2. Experimental

2.1. Materials

The solvents used in the present work were procured from E. Merck (Mumbai, India). The ODS C₁₈ cartridges were obtained from J&W Scientific (USA). The diesel was purchased from a local petrol filling station (Bharat Petroleum, Hyderabad, India). The compounds *O,O*-diisopropyl *N,N*-diethylphosphoramidate (**1**), *O,O*-dipropyl *N,N*-diethylphosphoramidate (**2**), and *O,O*-dibutyl *N,N*-diethylphosphoramidate (**3**) were prepared in the laboratory [27].

2.2. Preparation of standard solutions

Stock solutions of concentration 1000 ppm (w/v) of each compound (**1–3**) were prepared in 10 ml of methanol. The matrix was prepared by adding 10 µl of diesel in 1 ml of hexane to provide a strong hydrocarbon background and the matrix was spiked with compounds **1–3** by using the appropriate volumes of corresponding stock solutions so that the concentration of each compound is 10 ppm. The matrix in hexane without spiked compounds was used as the blank sample.

2.3. Solid-phase extraction

The ODS C₁₈ cartridge was pre-conditioned by passing through 2 ml methanol and 2 ml deionized water. One milliliter of the organic matrix sample spiked with the target compounds was concentrated under gentle nitrogen purging to a final volume of 50 µl. The sample was then mixed with 1 ml of water and sonicated for 1 min to result in an emulsion. The emulsion was immediately passed through the pre-conditioned SPE cartridge. The cartridge was then washed with 600 µl of hexane. The contents in the cartridge were finally eluted with 2.5 ml neutral methanol. The methanol

extract was concentrated by gentle nitrogen purging and re-dissolved in methanol to make a final volume of 1 ml. One microliter of the sample was injected into the GC–MS system operating in EI and CI modes.

2.4. GC–MS analysis

The GC–MS analysis was carried out on an Agilent 6890 GC system equipped with a 5973 N mass-selective detector (Agilent Technologies, USA). A HP-5 (Agilent Technologies) column of 30 m × 0.25 mm internal diameter and 0.25 µm thickness was used for the method development and a DB-1701 (Agilent Technologies) column of similar dimensions was used during 14th proficiency test conducted by the OPCW. The oven was programmed from an initial temperature 50 °C hold for 2 min and to the final temperature 280 °C at the rate of 10 °C/min. The final temperature hold up time was 5 min. Helium at the rate of 1 ml/min was used as the carrier gas in constant flow mode. The inlet and interface temperatures were kept at 280 °C. The EI source was operated at 230 °C and the quadrupole temperature was 150 °C in EI mode. The CI source was operated at 150 °C and the quadrupole temperature was 106 °C in CI mode. Isobutane was used as reagent gas maintained at 2 psi inlet pressure (1 psi = 6894.76 Pa) and 20% of total flow was introduced into the ion source. The MS was scanned from *m/z* 30 to 600 in EI mode and from *m/z* 60 to 600 in CI mode, respectively. The sample was injected in split mode at a split ratio of 5:1.

3. Results and discussion

Solid-phase extraction is generally used for extracting organic compounds from aqueous samples. In the present work, we used the SPE method to extract the scheduled compounds of CWC from the organic samples contaminated with high hydrocarbon background. Three compounds of schedule 2 precursors, namely *O,O*-diisopropyl *N,N*-diethylphosphoramidate (**1**), *O,O*-dipropyl *N,N*-diethylphosphoramidate (**2**), and *O,O*-dibutyl *N,N*-diethylphosphoramidate (**3**) are used as standard test compounds to study the extraction method. The compounds **1–3** are spiked at a level of 10 ppm each in 1 ml hexane containing 1000-fold hydrocarbon mixture, diesel (organic liquid). The hydrocarbon mixture, diesel in hexane is taken as blank sample. The total ion chromatograms (TICs) obtained from the direct GC–MS analysis of the organic liquid and the blank samples are shown in Fig. 1. The TICs of both organic liquid and blank are looking similar and show many peaks due to hydrocarbons and the spiked compounds are completely masked. The peaks of spiked chemicals can only be seen in extracted ion chromatograms (EICs). The resulted mass spectra of the compounds **1–3** cannot be identified unambiguously due to interfering peaks from co-eluting hydrocarbons. The organic liquid sample is then

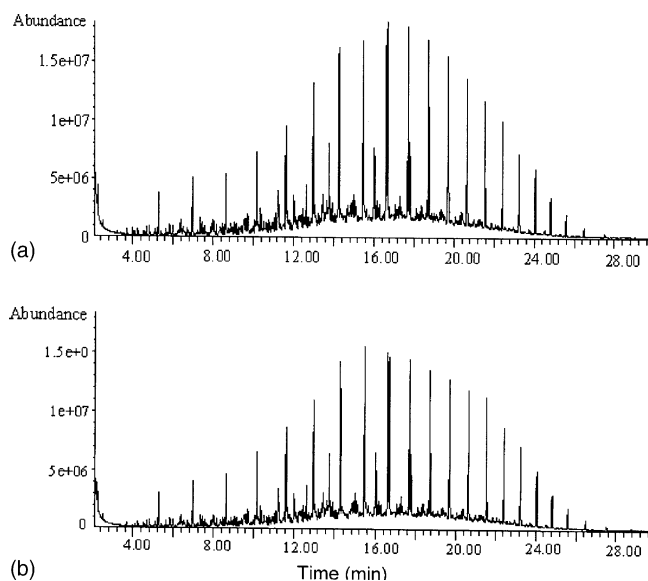


Fig. 1. Total ion chromatogram obtained from GC–EI–MS analysis of: (a) spiked organic liquid; (b) blank.

subjected to SPE analysis as described in Section 2. The organic matrix sample spiked with target compounds was concentrated to 50 μl and to that 1 ml of water was added prior to loading on the ODS C_{18} cartridge. At this point, diesel or hexane would not be soluble in water as the solubility of hexane in water is only 0.001%. In order to load entire sample on cartridge and to minimize the sticking of hexane on the walls of vessel, the sample was sonicated for a minute to result in an emulsion. The resulted emulsion was loaded on the preconditioned cartridge and then washed with 600 μl of hexane. The GC–EI–MS analysis of hexane layer showed the absence of spiked chemicals but enriched with diesel background (Fig. 2). The contents in cartridge were then eluted with methanol as described in Section 2. The methanol fraction is subjected to the GC–MS analysis and the TIC is shown in Fig. 3. The peaks of the spiked compounds are now clearly visible in TIC and the hydrocarbon background is significantly reduced. It clearly demonstrates that the hydrocarbon background is decreased during SPE process and not because of partitioning between the diesel–hexane–water prior to SPE. The extracted

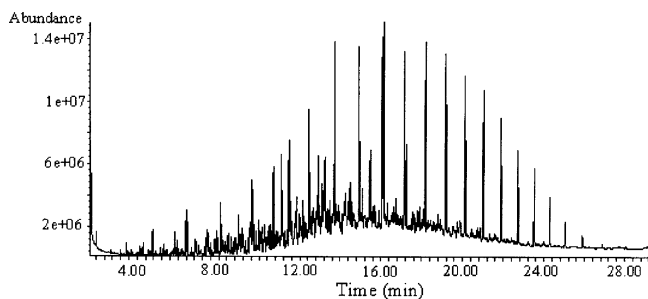


Fig. 2. Total ion chromatogram obtained from GC–EI–MS of hexane fraction collected during SPE process of spiked organic liquid sample.

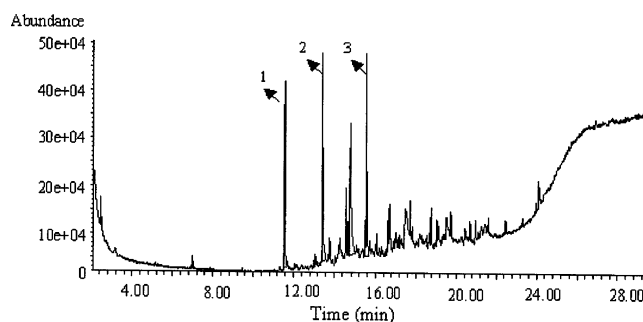


Fig. 3. Total ion chromatogram obtained from GC–EI–MS analysis of the spiked organic liquid after SPE.

ion chromatogram for each compound is very much clear and free from hydrocarbon peaks. The EI mass spectra obtained for compounds 1–3 from the GC–MS analysis of organic liquid after SPE are very clear and comparable to the corresponding standard spectra. The mass spectra of the compounds 1–3 obtained from the GC–MS analysis of organic liquid before and after SPE are presented in Figs. 4–6, respectively.

The recovery of compounds 1–3 from the SPE is calculated from peak areas measured from ion chromatogram of selected ion (m/z 138) obtained during GC–MS analysis of organic liquid after SPE and the standard solutions of compounds 1–3. For this purpose, the methanol fraction from SPE was concentrated and again reconstituted up to 1 ml with methanol, as described in Section 2, to obtain equal final volumes of sample during repeated experiments. The recovery for compounds 1–3 is found to be consistent with load during SPE method and the percent recovery values ($n = 5$)

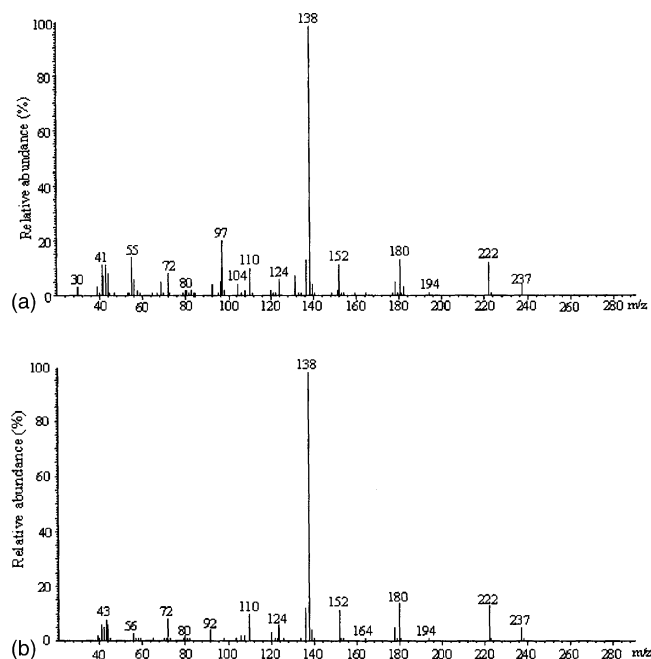


Fig. 4. EI mass spectrum of compound 1 obtained from GC–MS analysis of spiked organic liquid: (a) before SPE; (b) after SPE.

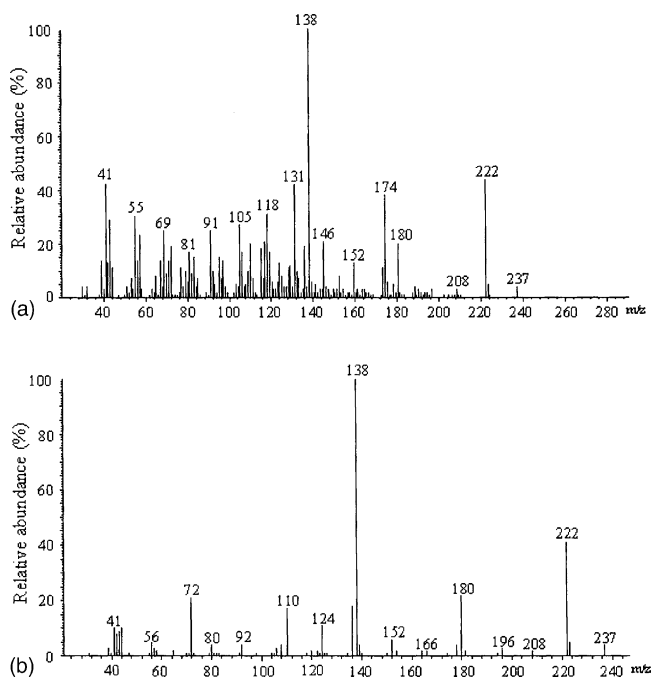


Fig. 5. EI mass spectrum of compound **2** obtained from GC–MS analysis of spiked organic liquid: (a) before SPE; (b) after SPE.

are 73.2 ± 9.5 for **1**, 85 ± 10.3 for **2** and 83.4 ± 8.2 for **3**. Use of large volumes (>1 ml) of hexane in SPE method reduced the recovery of samples by 5–10%. The minimum detection limit of compounds **1–3** after SPE has also been tested. For these experiments the methanol extract from SPE is further concentrated to $100 \mu\text{l}$ by nitrogen purging and $1 \mu\text{l}$ of sample injected into GC–MS. The samples could be detected in

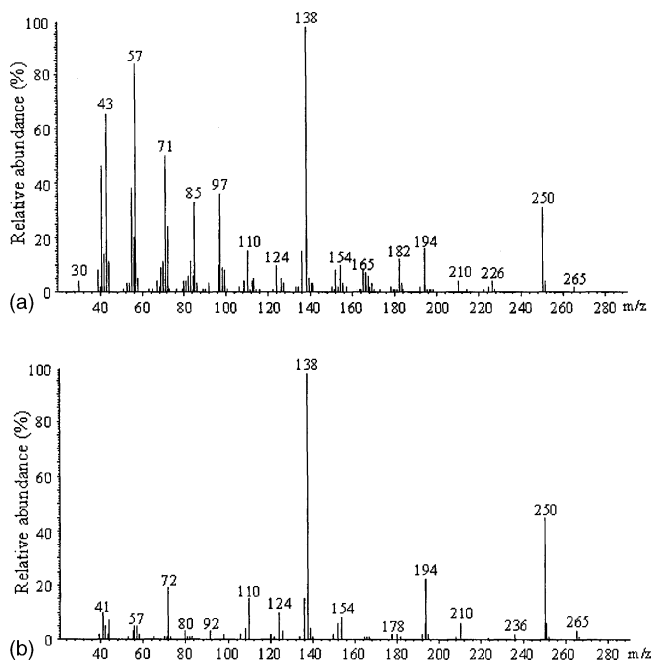


Fig. 6. EI mass spectrum of compound **3** obtained from GC–MS analysis of spiked organic liquid: (a) before SPE; (b) after SPE.

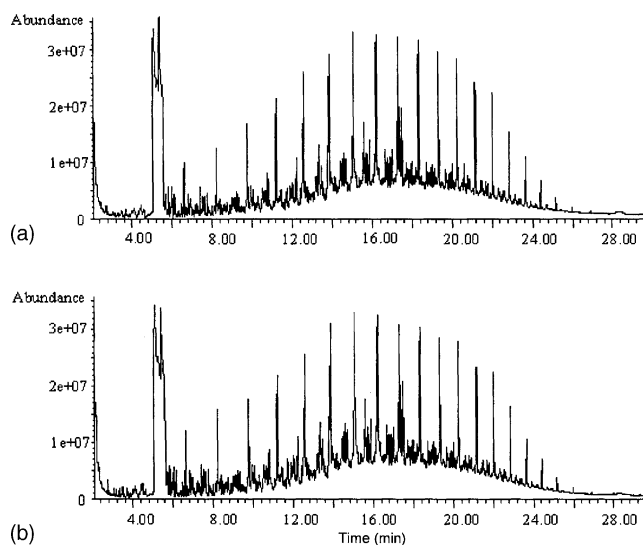


Fig. 7. Total ion chromatogram obtained from direct GC–EI–MS analysis of OPCW test organic samples: (a) blank; (b) sample.

full scan mode when the organic liquid spiked with 1 ppm of test samples are subjected to SPE. It was found that the background peaks are dominating over the sample peaks in full scan mode at the concentration of less than 1 ppm level. This demonstrates that the samples can be recovered from cartridge at low levels. Thus, the present SPE technique can be successfully used for the identification of CWAs present at trace level in complex hydrocarbon matrices.

This method has been successfully employed for the analysis of organic sample during the 14th official proficiency test conducted by the OPCW. The TICs obtained from the direct GC–MS analysis of the organic sample and the corresponding blank showed similar chromatograms and were dominated with the hydrocarbon peaks that were used as matrix (Fig. 7). After careful analysis of TICs of sample and blank, the presence of few spiked chemicals were detected. However, the mass spectra contained background peaks that are not comparable with library data for unambiguous identification. Concentration of the sample was found to increase the complexity in identification. Hence, the SPE method as described above was used to remove the hydrocarbon background. The TICs obtained from GC–EI–MS analysis of OPCW test samples after SPE treatment are given in Fig. 8. The TIC of sample showed clearly distinguishable peaks corresponding to spiked chemicals. The four major peaks were confirmed as diethyl *N,N*-diethylphosphoramidate, ethyl *N,N*-dimethylphosphoramidocyanidate (tabun), cyclohexylethylphosphonofluoridate and propyl *N,N*-2-diisopropylaminoethylphosphonothiolate. Two other minor peaks were found to be propylmethylphosphonofluoridate and fluorotabun. The EI mass spectrum of tabun obtained from sample before and after SPE is presented in Fig. 9 as an example. The molecular masses weights of these compounds were confirmed with the CI technique which gave dominant $[M + H]^+$ ions in their CI mass spectra. It has to be pointed

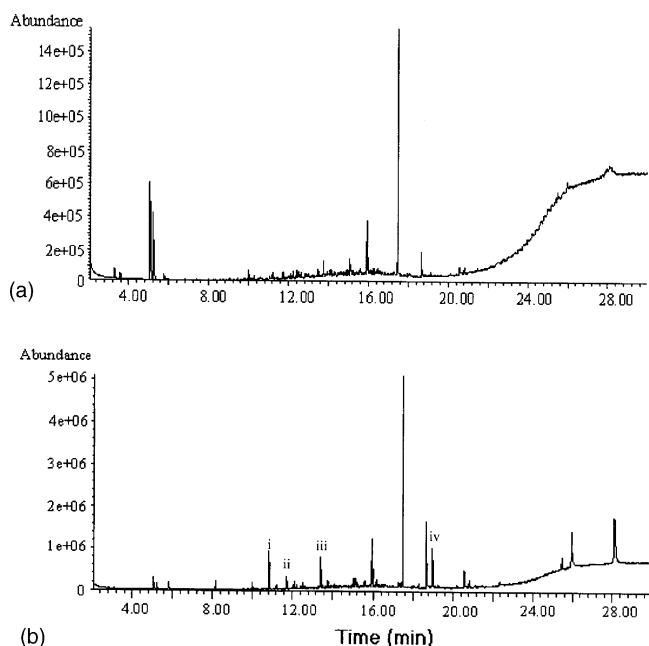


Fig. 8. Total ion chromatogram obtained from GC–EI–MS analysis of OPCW test organic samples: (a) blank after SPE; (b) sample after SPE. (i) *O,O*-Dimethyl *N,N*-diethylphosphoramidate; (ii) tabun; (iii) cyclohexylethylphosphonofluoridate; (iv) propyl *N,N*-2-diisopropylaminoethylphosphonothiolate.

out that the other two minor peaks were not visible in the original sample without any purification step by the analysis either with NPD or with MS. The SPE technique has definitely enriched the target compounds from the original solution and hence they were easily detectable after SPE.

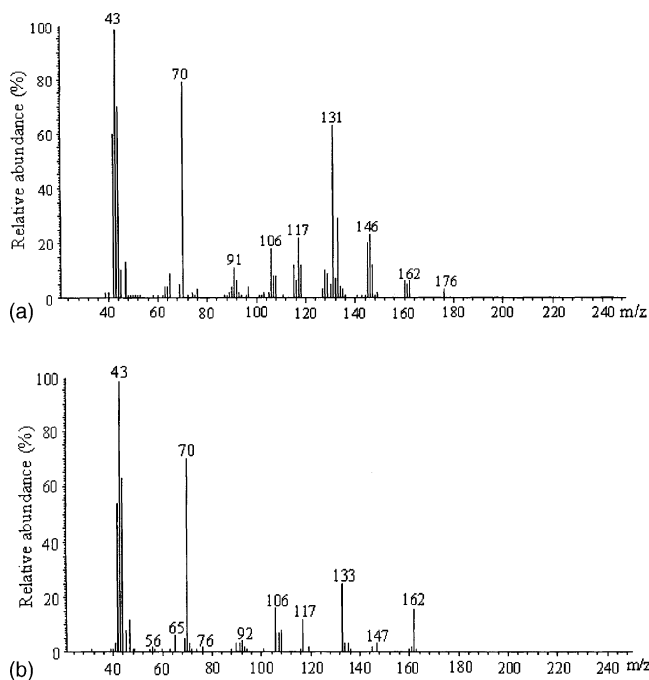


Fig. 9. EI mass spectrum of tabun obtained from GC–EI–MS analysis of OPCW test sample: (a) before SPE; (b) after SPE.

4. Conclusions

Generally it is difficult to detect and identify the schedule compounds of CWC by direct GC–EI–MS analysis in presence of dominant masking/background components, especially in complex hydrocarbon background. We have adopted an SPE method successfully to detect and identify CWAs present in a complex organic background. The commercially available ODS C_{18} cartridges are used for this method. The SPE method used involves selective usage of solvents to elute the compounds of interest from cartridge with a little hydrocarbon background. Three *N,N*-dialkyl *O,O*-dialkylphosphoramidates spiked at low levels (10 ppm) along with high diesel content (1000-fold) as hydrocarbon background in hexane is used as test sample to apply the SPE method. The TIC obtained from GC–EI–MS analysis of the sample after SPE is very clear and resulted in clean EI mass spectra to identify the spiked compounds unambiguously. The recovery of sample from SPE is measured to be 70–85% and samples of 1 ppm concentration are found to be recoverable from the cartridge that can be detected by GC–EI–MS. The same SPE method was used successfully during the 14th proficiency test conducted by OPCW to identify four CWAs in an organic liquid sample containing strong hydrocarbon background.

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