DEVELOPMENT OF A SPME-GC-MS BASED METHOD FOR ANALYSIS OF ORGANOCHLORINATED SMOKE AGENTS IN SOIL AND ITS APPLICATION IN A FORMER MILITARY SITE SAMPLES

(Pembangunan Kaedah Analisis Berasaskan SPME-GC-MS untuk Analisis Agen Asap Organoklorin di dalam Tanah dan Kegunaannya dalam Analisis Sampel di Tapak Tanah Pemulihan Kem Tentera)

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Abstract

Smoke agents contained chlorinated compounds with a high environmental impact such carbon tetrachloride and hexachloroethane have been used for a long time by North Atlantic Treaty Organization (NATO). The aim of this paper is to present an analytical method for the determination of these organochlorinated compounds based on Solid Phase Microextraction (SPME) and Gas Chromatography Mass Spectrometry (GC-MS) in optimizing the temperature and the time of fiber exposure. This method has been compared with the reference method based on static headspace coupled with GC-MS. The SPME method proves performance was improved especially in term of sensibility respect to the reference one. The SPME method has been successfully applied to real samples from a remediation soil of former military site.

Keywords: solid phase micro extraction, smoke agents, hexachloroethane, carbon tetrachloride

Abstrak

Agen asap yang diketahui mempunyai impak terhadap kesan alam sekitar mengandungi sebatian berklorin seperti karbon tetraklorida dan heksakloroetana telah diguna untuk tempoh masa yang lama oleh Pertubuhan Perjanjian Atlantik Utara (NATO). Kajian ini dilakukan bertujuan membangunkan satu kaedah analisis bagi penentuan kehadiran sebatian organoklorin berdasarkan teknik pengekstrakan mikro fasa pepejal (SPME) dan gas kromatografi- jisim spektrometri (GC-MS) bagi mengoptimumkan suhu dan masa pendedahan serat. Kaedah ini telah dibandingkan dengan kaedah rujukan berdasarkan ruang kepala statik yang dilengkapi dengan GC-MS. Kaedah SPME ini membuktikan prestasi yang lebih baik terutama dari segi kederiaan berbanding kaedah rujukan. Kaedah SPME telah berjaya digunakan untuk analisis sampel sebenar dari tapak tanah pemulihan kem tentera.

Kata kunci: pengekstrakan mikro fasa pepejal, agen asap, heksakloroetana, karbon tetraklorida

Introduction

Chemicals and materials used in military activities have a remarkable environmental effect. The remediation of contaminated military sites can be a real issue since the step of characterization, difficulties in finding standards, reference materials and affordable analytical methods and moreover for the traditional military secrecy. The most dangerous class of molecules used in military field are the chemical warfare agents [1-5]. Military range and impact area can be contaminated by explosives [6] and heavy metals [7]. A less known class of military pollutants are organochlorinated molecules used in the past for smoke generation. The smoke bombs are a tool used by military personnel to create smokescreen and hide troop movements to the enemy or for signalling. There were two main class of smoke bombs: smoke candles similar to fireworks and smoke grenades built with metal canisters, are both used to create a thick cloud of smoke for up to several minutes. These devices before the Eighties were made with a high percentage of chlorinated substances. Smoke bombs contained also other compounds such zinc powder, zinc oxide and asphalt but these materials were largely less toxic then the chlorinated ones that are the most environmentally concern. Carbon tetrachloride and hexachloroethane were the most used organochlorinated substances for this purpose and these compounds are now considered by International Agency for Research on Cancer (IARC) as "possible carcinogenic to humans" [8, 9].

The Italian Istituto Superiore di Sanità (ISS) identified a General Population Exposure Limit for both compounds from reference toxicological data. For carbon tetrachloride, it indicates a limit of 100 μg/kg and for groundwater it gave a limit of 0.15 μg/l. Meanwhile for hexachloroethane, there is an estimation of 500 μg/kg in soil and 0.005 μg/l for groundwater. It is important to identify the legal references limits to set the limits of detection that the analytical methods should achieve.

The reference method of sample preparation for volatile substances analysis such as carbon tetrachloride in soil is the Head Space (HS) (EPA 5021a) combined with GC-MS according to EPA 8260c as reported by USACERL [10]. An analysis of Hexachloroethane proposed by USACERL [10] recommends various sample preparation and analytical methods. For example sample preparation used solvent extraction followed by clean up with Florisil (EPA 3620) or with Gel Permeation Chromatograpy (GPC) (EPA 3640) then the analysis was carried out with GC-MS as stated in method EPA 8270 or 8260.

The aim of this work is to validate an analytical method using headspace Solid Phase Micro Extraction (SPME) and to compare it with the performance of reference static headspace technique on both analytes by exploiting its performance. SPME is a sample preparation method which well known in environmental field [11] since it have been applied to different analytes and matrices such pesticide [12] or Polycyclic Aromatic Hydrocarbon (PAH) [13] in water or other non-metal analysis [14]. The SPME based methods also have been applied for analysis of carbon tetrachloride in water and soil [15] and hexachloroethane in gas from plasma pyrolysis [16] but to the best of our knowledge, it never have been applied to analysis of hexachloroethane in soil.

Materials and Methods

Reagents and Standards

Standard materials of pure carbon tetrachloride (CAS 56-23-5) and hexachloroethane (CAS 67-72-1) were purchased respectively from Supelco and Sigma Aldrich. Stock solutions of both compounds were prepared at 1000 μg/ml concentration using acetone. Two secondary solutions were prepared (20 μg/ml and 5 μg/ml) to spike in the soil samples. Deuterated benzene (CAS 1076-43-3) from Sigma Aldrich was used as internal standard at 10 μg/ml concentration in acetone, to normalize the instrumental response in different injections. PolyDiMethylSiloxane (100 μm PDMS) fiber purchased from Supelco was chosen for this study. This specific kind of fiber is recommended by Supelco Application note [17] for non-polar volatile compounds because it gives an higher instrumental response.

Instrument

An Agilent GC 7890, MSD 5975 was used equipped with a 60 m column SPB1 with oven programmed: initial temperature of 45 C° for 10 minutes then ramp of 15 degrees for minute till 240 C° maintained for 5 minutes. Transfer line temperature was 280 C°. The only difference was the usage of different specific liner for static headspace and for SPME method and a different injector temperature: 220 C° for static headspace and 250 °C for SPME. The acquisition is Single Ion Mode (SIM) with masses for Carbon Tetrachloride m/z 117 – 119/121 and for

Hexachloroethane m/z 117 – 119,201 with first mass used for quantification and the other two used for confirmation.

Samples Preparation for Methods using Static Headspace
The sample preparation for static headspace method based on EPA 5021a: 2 g of soil with 30% w/w of water are placed in a 22 ml vial for HS analysis and 10 μl of standard in acetone at concentration level 20 μg/ml of carbon tetrachloride and hexachloroethane was added to obtain a spiked soil at 100 μg/kg concentration. Then, 10 ml of saturated sodium chloride solution and 1 ml of phosphate buffer were added as reported in EPA method. This is common practice in environmental analysis, since soil samples with different amount of soluble salts or with rocks giving a different pH became much more similar and to reduce the matrix effect in following analysis. An internal standard solution of deuterated benzene in acetone also was added. The extraction time was 50 minutes and the temperature was set up at 80C° as reported in static headspace EPA method.

Samples Preparation for Method using Headspace SPME
5 g of soil sample with 30% w/w of water are placed in a 40 ml vial with pierce able septum cap and 10 μl of standard in acetone at 5 μg/ml concentration was added to spike the samples to obtain a final concentration of 10 μg/kg. To improve repeatability, 20 ml of saturated sodium chloride solution and 2 ml of buffer phosphate were added, to reduce the matrix effect as reported for samples for headspace analysis. Then, the internal standard solution of deuterated benzene in acetone was added. The SPME fiber was introduced into the vial by piercing the septum and exposed in the headspace of the sample. After reach exposure time, the fiber is retracted and moved to the GC injector where is thermally desorbed.

Results and Discussion
Method Evaluation using Static Headspace
For static headspace method, the uncertainty was estimated as standard deviation of the instrumental response of six replicate spiked at the same concentration. The linearity of method was calculated as coefficient of determination (R^2) of instrumental response, spiking the soil samples at 4 different concentrations (10, 50, 100, 200 μg/Kg). The Limit of Quantification (LOQ) was calculated as the signal corresponding 10 times to the noise, obtaining both signal and average noise from spiked samples chromatograms. In spite of information reported by USACERL [10] also static headspace method performed for both analytes, the performance is necessary in the work of characterization of a contaminated site because the LOQs are well below the legal exposure limit that come from the literature for both the analytes. The LOQ of hexachloroethane is about two order of magnitude higher respects to carbon tetrachloride It also noted that probably analytical method with a lower LOQ for hexachloroethane is necessary only for groundwater's analysis that with exposure limit much lower respect the carbon tetrachloride one. The linearity and the uncertainty of the method for both analytes fits the purpose of this work in the tested range. The results are summarized in table 1.

Table 1. Static headspace method's performance

Analyte	RSD (%)	R^2	LOQ (μg/kg)
Carbon Tetrachloride	12%	1	0.03
Hexachloroethane	6%	0.99	1.6

Optimization of Samples Preparation Methods with SPME
Extraction time was optimized at a fixed temperature of 60 °C. A time profile was obtained (Figure 1) show the correlation between the instrumental response (ion m/z 117) and the times of exposure of the fiber. The times tested are 10, 20, 30, 45 and 60 minutes. These data show that is sufficient to wait 30 minutes to obtain the maximum of instrumental response for both analytes, after maximum time there is no more a significant improvements for both of them, and then this time of exposure is used in the following work.
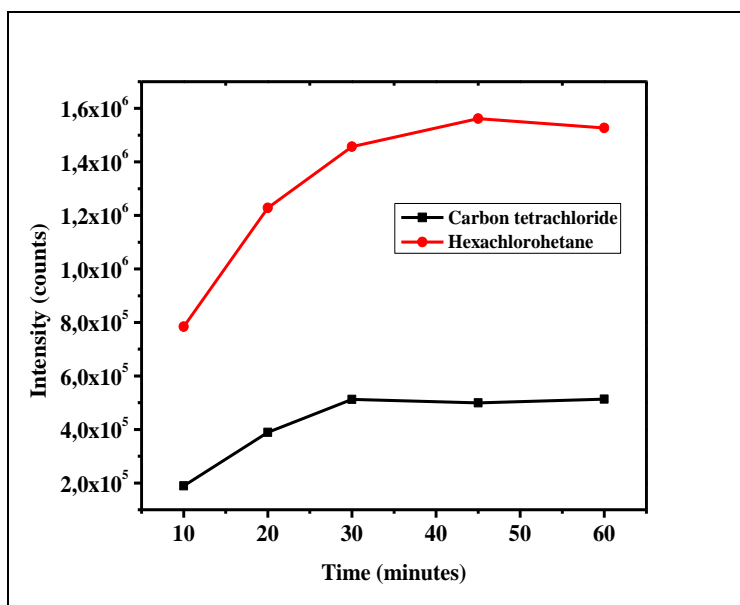
Figure 1. Correlation between time and detector response (ion m/z 117)

A temperature profile was obtained (Figure 2) show the correlation between the instrumental response (ion m/z 117) and the times of exposure of the fiber. The temperatures chosen for tests are 20, 40, 60 and 80 °C. These data show that with a temperature higher than 60 C° there are not significant improvement in instrumental response for both analytes, and then this temperature have been used in the following work.
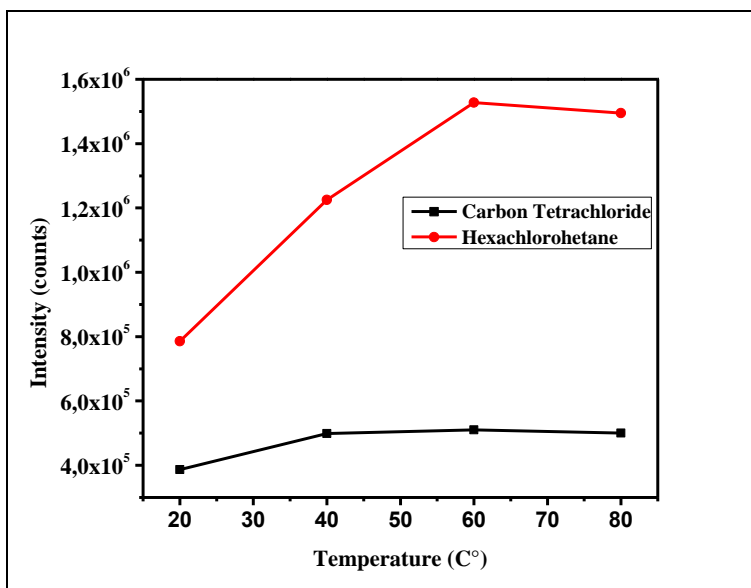

Figure 2. Correlation between temperature and detector response (ion m/z 117)

Method evaluation of optimized condition has been evaluated. The uncertainty has been estimated as standard deviation of the instrumental response of six replicate spiked at the concentration of 10 µg/Kg. The linearity of method was calculated as coefficient of determination (R^2) of instrumental response, spiking the soil at 4 different levels (1, 5, 10, 20 µg/Kg). The Limit of Quantification (LOQ) was calculated as the signal corresponding 10 times to the noise, obtaining data of noise and signal from chromatogram of spiked samples. The results of method evaluation are summarized below in Table 2. A chromatogram corresponding to a spiked soil with the concentration of 10 µg/Kg is shown in Figure 3.

Table 2. Headspace SPME methods performance

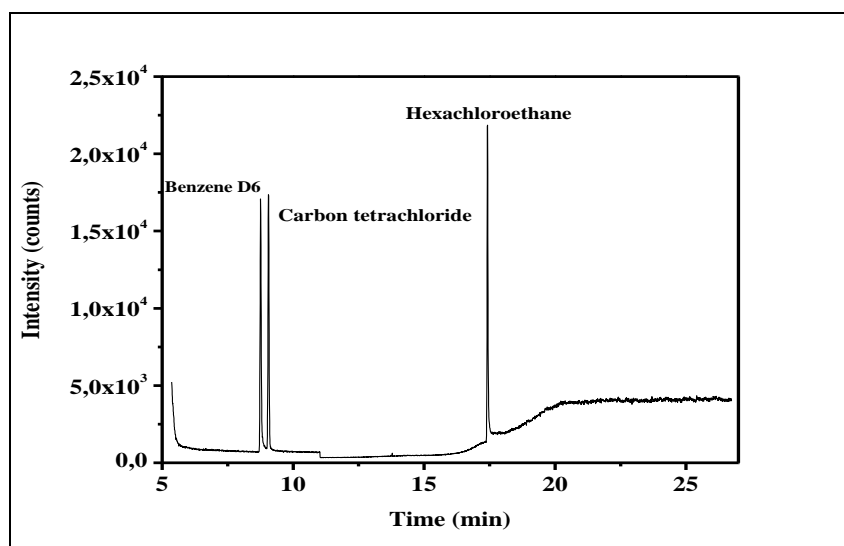| Analyte	RSD (%)	R^2	LOQ (µg/Kg)
Carbon Tetrachloride	13%	0.95	0.002
Hexachloroethane	16%	0.98	0.001

Figure 3. Chromatogram of a standard at 10 µg/Kg

Headspace SPME and static headspace methods both fits the performance needed. SPME method has significantly higher sensitivity, especially for hexachloroethane, with a LOQ that obtained is three orders of magnitude lower respect static headspace. The method based on SPME appears to have linearity of response slightly lower and uncertainty slightly higher compared with method based on static headspace. In application of optimized method, samples from former military plant in central Italy were collected. The abandoned site opened in the Thirties during the Fascist regime as research centre and production plant for chemical warfare in an area of about thirty acres and this remain its main role until the end of World War II. In this period, it was used especially for charging of airplane bombs and artillery shells with CWA. Also after the end of War, Italian Army also used to store old chemical warfare agents in this plant and used it for charging of smoke agent shell. About 50 tons of hexachloroethane and 14 tons of carbon tetrachloride result in the inventories the very moment of its phasing out in 1995. Real samples were collected during 2013 for the characterization of the site that goes hand in hand with military clearness, which involves the inspection and removal of a number of magnetic anomalies identified in a previous phase of geophysical investigation. In addition of the expected eighteen excavations of a real averaged samples, for a total of

800 m^2, have been collected also seven punctual samples corresponding to textural, chromatic or olfactory anomalies of soil.

The SPME analytical method has been applied to every a real-averaged samples without finding evidence of organochlorinated smoke agents. Also major parts of point samples were blank with a single exception: the P-A sampled in M5 area, shown in Figure 4, in which quantify was contained only carbon tetrachloride. Sample was taken at the bottom of an excavation in a layer of anthropogenic origin of pale yellow colour. The calculated concentration of carbon tetrachloride varied until a maximum calculated concentration is 53 μg/Kg. A chromatogram of real sample is reported in Figure 5. However, the concentration of carbon tetrachloride in this sample in every replicates is largely below the toxicity limit (100 μg/Kg).

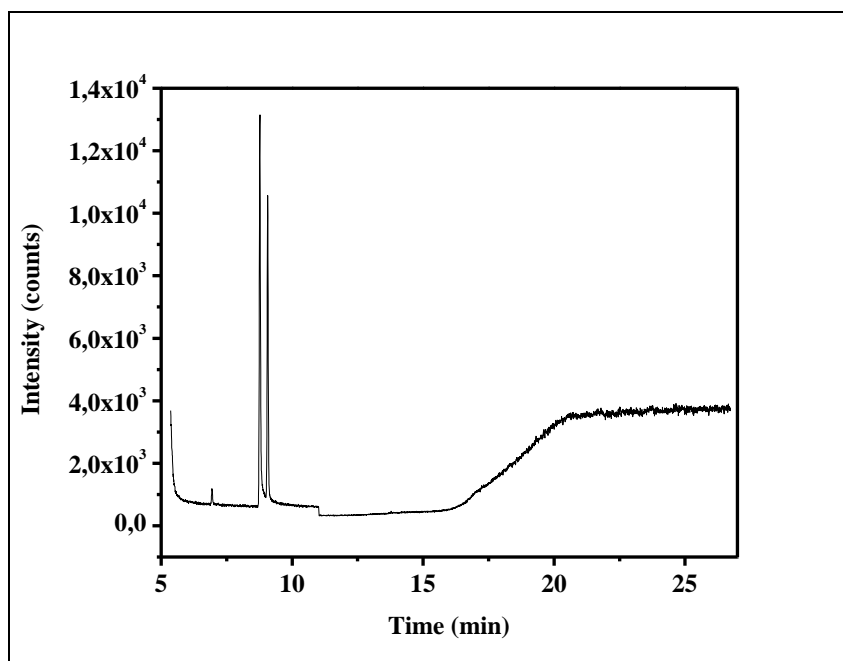Figure 4. Remediation area of military site

Figure 5. Chromatogram of real sample analysis at PA – M5

Conclusion

In this work, it was demonstrated that proposed analytical method based on SPME headspace technique is definitely more sensitive respect to the reference one based on static headspace. The SPME method has been applied to complex real environmental matrices, without samples preparations, reducing the costs of the characterization of the sites involved in remediation. A further interesting aspect is that this technique not only offers excellent performance and efficiency, but it is also highly recommendable for its safety and environmental friendliness [18] thanks to the fact that no additional solvent is required. This is an importance aspect for the community of analytical chemist in the recent years have been focused on research in developing and applying analytical methods with a lower environmental impact, that so called as green analytical chemistry [19, 20, 21].

Acknowledgement

References

1. Tørnes, J. A., Opstad, A. M. and Johnsen, B. A. (2006). Determination of organoarsenic warfare agents in sediment samples from Skagerrak by gas chromatography-mass spectrometry. *Science of the Total Environment* 356(1) : 235-246.
2. Alcaro, L., Torre, C. D., Petochi, T., Sammarini, V., Matiddi, M., Corsi, I., Baroni, D., Giordano, P., Marino, G. Focardi, S. and Amato, E. (2012). Studies on Environmental Effects of Underwater Chemical Munitions in the Southern Adriatic Sea (Mediterranean Sea). *Marine Technology Society Journal* 46(3): 10-20.
3. Pitten F.A., Thurow K., Koch A., Kramer A. (2000). Chapter 6 - Chemical analysis of organoarsenic based chemical warfare agents in the environment Trace elements their distribution and effect on environment – B. Markerf and K. Friese Editor Elsevier.
4. Hanaoka, S., Nagasawa, E., Nomura, K., Yamazawa, M. and Ishizaki, M. (2005). Determination of diphenylarsenic compounds related to abandoned chemical warfare agents in environmental samples. *Applied Organometallic Chemistry*, 19(2): 265-275.

5. Leermakers, M., Baeyens, W., De Gieter, M., Smedts, B., Meert, C., De Bisschop, H. C., Morabito R., and Quevauviller, P. (2006). Toxic arsenic compounds in environmental samples: Speciation and validation. *TrAC Trends in Analytical Chemistry*, 25(1): 1-10.

6. Jenkins, T. F., Hewitt, A. D., Grant, C. L., Thiboutot, S., Ampleman, G., Walsh, M. E., Ranney T. A., Ramsey C. A., Palazzo A. J. and Pennington, J. C. (2006). Identity and distribution of residues of energetic compounds at army live-fire training ranges. *Chemosphere*, 63(8): 1280-1290.

7. Massol-Deyá, A., Pérez, D., Pérez, E., Berrios, M., & Díaz, E. (2005). Trace elements analysis in forage samples from a US Navy bombing range (Vieques, Puerto Rico). *International Journal Of Environmental Research and Public Health*, 2(2): 263-266.

8. IARC Monographs (1979) 20: 371-399.

9. IARC Monograph (1999) 73: 295-306.

10. Nam, S. I., Walsh, M. E., Day, J. and Reinbold, K. A. (1999). Methods for Field Studies of the Effects of Military Smokes, Obscurants, and Riot-control Agents on Threatened and Endangered Species. Volume 4: chemical analytical methods USACERL Technical Report 56

11. Ouyang, G. and Pawliszyn, J. (2006). SPME in environmental analysis. *Analytical and Bioanalytical Chemistry*, 386(4): 1059-1073.

12. Ferrari, R., Nilsson, T., Arena, R., Arlati, P., Bartolucci, G., Basla, R., Cioni, F., Del Carlo, G., Dellavedova, P., Fattore, E., Fungi, M., Grote, C., Guidotti, M. Morgillo, S., Muller, L. And Volante, M. (1998). Inter-laboratory validation of solid-phase microextraction for the determination of triazine herbicides and their degradation products at ng/l level in water samples. *Journal of Chromatography A*, 795(2): 371-376.

13. King, A. J., Readman, J. W. and Zhou, J. L. (2004). Determination of polycyclic aromatic hydrocarbons in water by solid-phase microextraction gas chromatography–mass spectrometry. *Analytica Chimica Acta*, 523(2): 259-267.

14. Guidotti, M., Ravaioli, G., & Vitali, M. (1999). Selective Determination of Se $^{4+}$ and Se^{6+} using SPME and GC/MS. *Journal of High Resolution Chromatography*, 22(7): 414-416.

15. Chai, M., Arthur, C. L., Pawliszyn, J., Belardi, R. P. and Pratt, K. F. (1993). Determination of volatile chlorinated hydrocarbons in air and water with solid-phase microextraction. *Analyst* 118(12): 1501-1505.

16. Cubas, A. L. V., Carasek, E., Debacher, N. A. and de Souza, I. G. (2004). Use of solid-phase microextraction to monitor gases resulting from thermal plasma pyrolysis. *Chromatographia*, 60(1-2), 85-88.

17. Supelco Application Note 56 Fast Analysis of Volatile Organic Compounds by Solid Phase Microextraction/Capillary GC available on line https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Supelco/Application_Notes/4602.pdf

18. Wardencki, W., Curyło, J. and Namieśnik, J. (2007). Trends in solventless sample preparation techniques for environmental analysis. *Journal of Biochemical and Biophysical Methods* 70(2): 275-288.

19. Armenta, S., Garrigues, S. and De la Guardia, M. (2008). Green analytical chemistry. *TrAC Trends in Analytical Chemistry*, 27(6): 497-511.

20. Tobiszewski, M., Mechlińska, A. and Namieśnik, J. (2010). Green analytical chemistry—theory and practice. *Chemical Society Reviews*, 39(8): 2869-2878.

21. Tobiszewski, M., Mechlińska, A., Zygmunt, B. and Namieśnik, J. (2009). Green analytical chemistry in sample preparation for determination of trace organic pollutants. *TrAC Trends in Analytical Chemistry*, 28(8): 943-951.