Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
Journal of Chromatography A, 1038 (2004) 37–42
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Determination of pesticides in soil by liquid-phase microextraction and gas chromatography–mass spectrometry

Li Hou, Hian Kee Lee*

Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Singapore

Received 6 December 2002; received in revised form 12 February 2004; accepted 5 March 2004

Available online 9 April 2004

Abstract

Trace amounts of pesticides in soil were determined by liquid-phase microextraction (LPME) coupled to gas chromatography–mass spectrometry (GC–MS). The technique involved the use of a small amount (3 μl) of organic solvent impregnated in a hollow fiber membrane, which was attached to the needle of a conventional GC syringe. The organic solvent was repeatedly discharged into and withdrawn from the porous polypropylene hollow fiber by a syringe pump, with the pesticides being extracted from a 4 ml aqueous soil sample into the organic solvent within the hollow fiber. Aspects of the developed procedure such as organic solvent selection, extraction time, movement pattern of plunger, concentrations of humic acid and salt, and the proportion of organic solvent in the soil sample, were optimized. Limits of detection (LOD) were between 0.05 and 0.1 μg/g with GC–MS analysis under selected-ion monitoring (SIM). Also, this method provided good precision ranging from 6 to 13%; the relative standard deviations were lower than 10% for most target pesticides (at spiked levels of 0.5 μg/g in aqueous soil sample). Finally, the results were compared to those achieved using solid-phase microextraction (SPME). The results demonstrated that LPME was a fast (within 4 min) and accurate method to determine trace amounts of pesticides in soil.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Liquid-phase microextraction; Pesticides

1. Introduction

The presence of pesticides in the environment has caused great social and scientific concern. Traditionally, determination of trace levels of pesticide residues in aqueous samples relies on the use of liquid–liquid extraction (LLE) and solid-phase extraction [1,2]. These methods usually generate too much solvent waste and are labor- and time consuming. Recently, solid-phase microextraction (SPME) has been used for determination of pesticides in soil [3–6]. However, SPME fibers are relatively expensive, and can be fragile during extraction from soil samples. Usually, the use of SPME to determine pesticides in complex matrices (soil samples and food samples, etc.) requires some degree of sample clean-up, and membrane-protected SPME has also been developed to protect the fiber [7,8]. Another potential problem with SPME is sample carry-over between runs when SPME is coupled to gas chromatography (GC) [9].

Liquid-phase microextraction (LPME) is a fairly new method of sample preparation [10]. It is a miniaturized implementation of conventional liquid–liquid extraction (LLE) in which only microliters of solvents are used instead of several hundred milliliters in LLE. It is quick, inexpensive and can be easily automated. LPME has been used to preconcentrate compounds from aqueous samples [11–17]. We as well as others have previously applied LPME to the preconcentration of compounds from soil samples [18–20]. In the present study, a conventional microsyringe with a 1.3 cm length of hollow fiber attached to its needle was connected to a syringe pump to perform automated extraction of trace amounts of pesticides from soil samples. The hollow fiber prevented particles and large molecules in aqueous soil samples from being extracted. Thus, apart from analyte enrichment, the procedure also serves as a clean-up method.

Several pesticides in soil were studied by this method. Some important extraction factors, such as the portion of organic solvent in the soil–water slurry, the extraction solvent selection, extraction time, syringe speed, concentrations of humic acid, and salt were optimized. Finally, comparison of this method with SPME was also performed.

* Corresponding author. Tel.: +65-68742995; fax: +65-67791691.
E-mail address: chmleehk@nus.edu.sg (H.K. Lee).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.03.012

2. Experimental

2.1. Reagents and chemicals

2,5-Dimethylphenol, 2,3,5-trimethylphenol, Molinate and humic acid were bought from Fluka (Buchs, Switzerland). Lindane was obtained from Polyscience (Niles, IL, USA). 1,2,4,5-Tetrachlorobenzene, pentachlorobenzene, and hexachlorobenzene were purchased from AccuStandards (New Haven, CT, USA). Alachlor was bought from Supelco (Bellefonte, PA, USA). HPLC-grade methanol, toluene, analytical-grade carbon tetrachloride, and cyclohexane were from J.T. Baker (Phillipsburg, NJ, USA). 1-Octanol (>99.5%) was obtained from Merck (Darmstadt, Germany). Deionized water was produced on a Nanopure (Barnstead, Dubuque, IA, USA) water purification system. The Accurel Q3/2 polypropylene hollow fiber membrane (600 μm i.d., 200 μm wall thickness, 0.2 μm pore size) was purchased from Membrana GmbH (Wuppertal, Germany).

2.2. Soil sample preparation

Soil was sampled from the western region of Singapore. The soil was air-dried, pulverized, sieved to a grain size of 1 mm sequentially. Twenty-five weight percent of the particle size was below 250 μm. The soil pH measured (1 g soil in 5 ml water) was 5.8 [21]. The organic matter content was 5.45% [21]. The soil samples were extracted and analyzed by the present LPME method (as described below) to confirm that the analytes considered were absent and there were no other interfering compounds. Each pesticide was dissolved in methanol to obtain a standard solution with a concentration of 1.0 mg/ml. They were stored at 4 °C. Standard solutions at different concentrations containing the eight pesticides were prepared in methanol once every week and also stored at 4 °C. The standard solutions were added to the non-polluted soil (30 g) to make soil samples of several different analyte concentrations. The bulk of the solvent was slowly evaporated at room temperature by thorough manual shaking. These soil samples were allowed to dry in air in a fume hood for 24 h. One gram of soil to which were added acetone–water (4 ml total) at different ratios, served as soil samples for extractions. Soil samples were ultrasonicated for 5 min and stirred at 105 rad s^{-1} for 40 min before extraction. During all the following LPME experiments, the soil samples were stirred at 21 rad s^{-1}.

2.3. Instrumentation

All analyses were performed on a Shimadzu (Tokyo, Japan) QP5000 GC–mass spectrometry (MS) system. The GC was fitted with a DB-5 column (30 m × 0.32 mm i.d., 0.25 μm film thickness) from J&W Scientific (Folsom, CA, USA). Helium was used as the carrier gas at a flow rate of 1.7 ml/min. The following temperature programme was employed: initial temperature of 80 °C for 4 min; increased at 10°C/min to 150 °C, held for 1 min; then another increase at 5 °C/min to 200 °C; yet another ramp 30 °C/min to 250 °C, held for 2 min. The injector temperature was 280 °C and all injections were made in the splitless mode. A mass range of *m/z* 50–500 was scanned to confirm the retention times of the analytes. For determination of the pesticides, selected-ion monitoring (SIM) mode was performed. To confirm pesticide ions tentatively identified by SIM, one characteristic fragment ion was monitored in addition to the molecular ion: *m/z* 122,107 (2,5-dimethylphenol); 136,121 (2,3,5-trimethylphenol); 216,214 (1,2,4,5-tetrachlorobenzene); 290,181 (Lindane); 250,248 (pentachlorobenzene); 187,126 (Molinate); 284,286 (hexachlorobenzene); 269,160 (Alachlor) for each compound. The interface temperature was 270 °C. The peak areas were calculated based on the respective molecular ions. Two retention time windows were defined to increase the sensitivity of the MS analysis. Three molecular ions and three fragment ions in the first retention time window (retention times between 0 and 10 min), and five molecular ions and five fragment ions in the second retention time window (retention times between 10 and 20 min), were monitored.

2.4. LPME

A Harvard Apparatus (Holliston, MA, USA) model PHD 2000 syringe pump was used for automated extraction. A 10 μl microsyringe (SGE, Sydney, Australia) with a cone tip was used both for extraction and for injecting the extract into the GC–MS. The hollow fiber was of 1.3 cm length [16], cut manually prior to use. This length of hollow fiber allowed the use of ca. 3–4 μl of solvent for the microextraction. A new hollow fiber was used for every extraction and the used one was discarded. Briefly, LPME consists of the following steps: 3 μl of organic solvent (typically toluene) was withdrawn into the microsyringe. The needle tip was inserted into the hollow fiber and the assembly was immersed in the organic solvent for about 5 s to impregnate the pores of the hollow fiber with the organic solvent. Then, the organic solvent in the syringe was injected completely into the hollow fiber. The prepared fiber was removed from the organic solvent and subsequently immersed in the 4 ml aqueous soil sample. The microsyinge was then placed in position on the syringe pump, and the plunger was clamped by the pusher block and retaining bracket. The syringe pump was then switched on. The plunger was retracted at a speed of 0.5 μl/s to withdraw 3 μl of aqueous sample into the hollow fiber. After 4 s of dwelling (waiting) time, the plunger was depressed at the same speed to refill 3 μl of organic solvent into the fiber. The same dwelling time of 4 s was repeated. The above cycle was then repeated for a prescribed number of times. After the 3 μl analyte-enriched solvent was withdrawn into the syringe, the syringe pump was switched off. The syringe needle and hollow fiber was removed from the sample solution and the latter discarded. About 1 μl of analyte-enriched extract was discarded and the

remainder (2 μl) was injected directly into the GC–MS for analysis.

2.5. SPME

The SPME experiments [17] were performed using a manual SPME device (Supelco Inc., Bellefonte, PA, USA) equipped with 85 μm-thick fiber coated with polyacrylate adsorbent. The fiber was conditioned according to the supplier's recommendation. One gram soil sample to which were added 1.6 ml acetone and 2.4 ml water, was used for every extraction. The soil sample was then ultrasonicated for 5 min and was stirred for 40 min before extraction. The aqueous soil sample was extracted for 30 min at a stirring rate of 105 rad s^{-1}. After extraction, thermal desorption was performed in the GC injector at 280 °C for 3 min.

3. Results and discussion

3.1. Optimization of LPME

The mechanism of LPME used in our experiments has been described before [16]. Toluene, 1-octanol, carbon tetrachloride, and cyclohexane were tested as extraction solvents. The extraction was performed by using a 4 ml aqueous soil sample containing the eight pesticides at a concentration of 0.5 μg/g, over an extraction time of 3 min. Toluene gave the best extraction result among the four extraction solvents in terms of analyte peak areas (Fig. 1). This may be due to the strong compatibility between solvent and analytes (principle of *like attracts like*). Therefore, it was selected as the extraction solvent of choice.

Extraction time profiles were studied by extracting aqueous soil samples containing 0.5 μg/g of pesticides. All other parameters and conditions were the same as those mentioned above. The peak area counts were plotted as a function of

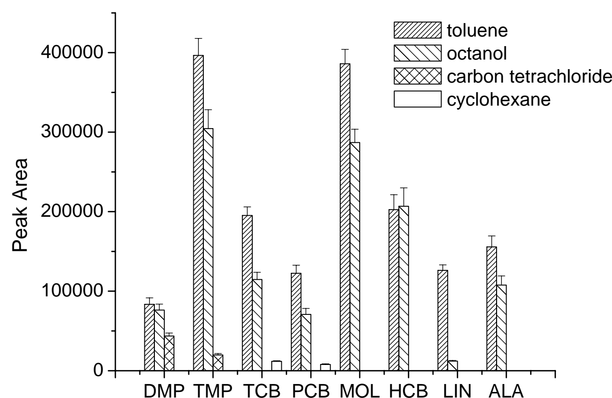

Fig. 1. Effect of extraction solvent on LPME efficiency ($n = 3$). Plunger movement speed: 0.5 μl/s; dwelling time: 2 s. Abbreviations: DMP, 2,5-dimethylphenol; TMP, 2,3,5-trimethylphenol; TCB, 1,2,4,5-tetrachlorobenzene; PCB, pentachlorobenzene; MOL, Molinate; HCB: hexachlorobenzene; LIN, Lindane; ALA, Alachlor.

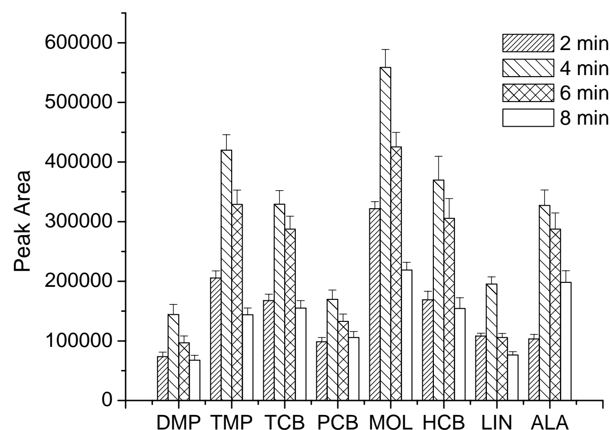

Fig. 2. Effect of extraction time on LPME efficiency ($n = 3$). Plunger movement speed: 0.5 μl/s; dwelling time: 2 s. See Fig. 1 for explanations of abbreviations.

extraction time (Fig. 2). As seen from Fig. 2, most pesticides investigated can reach the greatest response in 4 min. LPME is a process dependent on equilibrium rather than exhaustive extraction [16]. The extraction equilibrium was established very fast in this method. The fast extraction also ensured that no soil particles contaminated the extraction solvent. After 4 min, LPME efficiency was observed to decrease, probably because some of the toluene was lost, along with extracted analytes. Based on the above experiments, 4 min was fixed as extraction time.

The effect of the movement pattern of the plunger on the extraction was investigated. In our LPME process, the extraction was performed by automatically manipulating the plunger repeatedly in and out of the microsyringe barrel. Thus, fresh organic film and aqueous soil sample plug were involved in every extraction cycle. This is an advantage of this method. The plunger movement speed (sampling volume/withdrawal time = sampling volume/discharge time) and the dwelling time between plunger movement on extraction efficiencies were studied. First, setting the plunger movement speed at 0.5 μl/s, the dwelling time was varied to extract aqueous soil samples containing 0.5 μg/g of pesticides. Results are shown in Fig. 3. For most of the analytes (except hexachlorobenzene), the extraction efficiency was optimum when dwelling time was fixed at 4 s. With the dwelling time fixed at 4 s, we carried out separate experiments in which the plunger movement speed was varied. Peak areas, as measured by GC–MS, increased with the increase in plunger movement speed. Since 0.5 μl/s was the fastest speed at which the instrument could operate automatically, it was selected as the plunger movement speed for subsequent work.

The effect of the organic solvent content in aqueous soil samples on LPME efficiency was studied. It was found that when only water was added to the soil sample, it was difficult to extract the pesticides. In order to facilitate the release of the pesticides from the soil matrix, methanol, and acetone were added separately to the water–soil slurry at

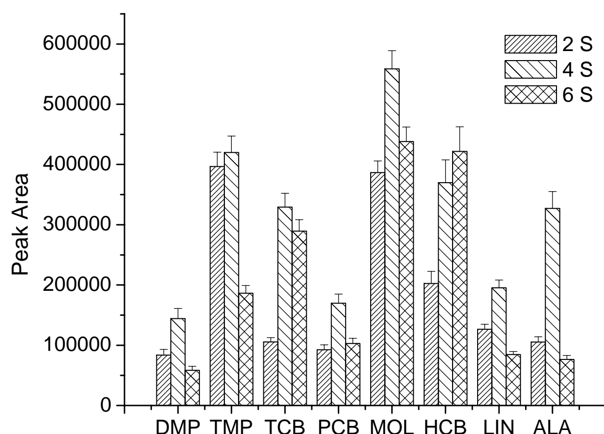

Fig. 3. Effect of dwell time on LPME efficiency ($n = 3$). Extraction time: 4 min. See Fig. 1 for explanations of abbreviations.

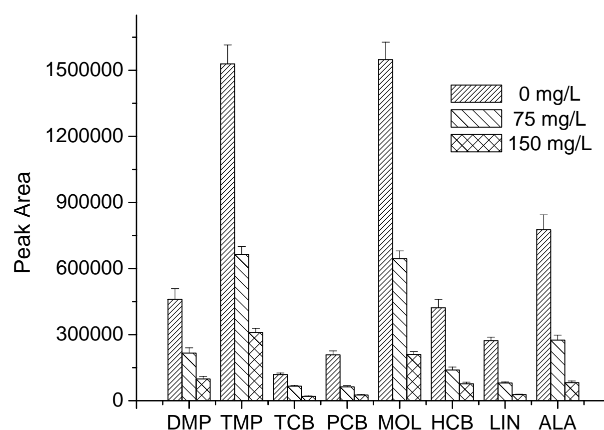

Fig. 5. Effect of addition of humic acid on LPME efficiency ($n = 3$). Final concentration of each analyte in spiked soil sample = 0.5 μg/g. See Fig. 1 for explanations of abbreviations.

proportions ranging from 20 to 50% and the extraction evaluated. When methanol was added to the soil matrix, there was no significant increase in extraction efficiency. However, the addition of acetone enhanced the extraction from the soil sample (Fig. 4). This might be because acetone could efficiently displace the pesticides from the soil active sites and into the water. When acetone (40%) was added to the soil sample, the extraction efficiency was significantly enhanced for most analytes except Molinate and pentachlorobenzene. Forty percent acetone was therefore used for subsequent experiments.

The effect of humic acid concentration on LPME efficiency was investigated by varying the concentrations in the range of 0–150 mg/l (Fig. 5). It was found that the extraction efficiency decreased, with increasing concentration of humic acid.

Finally, the effect of salt concentration on LPME efficiency was investigated by varying the concentration of NaCl between 10 and 30% (Fig. 6). The effect was basically negative for all analytes. For Molinate and 2,3,5-trimethylphenol, the extraction efficiencies decreased significantly with the addition of salt. For other analytes, the extraction efficiencies decreased slowly with the addition of salt. It was also observed that soil particles were easily drawn into the syringe when the concentration of NaCl was ca. 20%. Based on the above observations, it would seem that addition of NaCl offered no benefits to the extraction.

3.2. Method evaluation

3.2.1. Linearity, repeatability, and relative recoveries

Under the optimal LPME conditions, repeatability, relative recoveries, and the linearity of the method were investigated. Table 1 shows all the quantitative results of this method. The repeatability in peak areas was studied for six replicate experiments with pesticide concentrations of 0.2 μg/g. The relative standard deviations (R.S.D.) for most analytes were lower than 10% except for 2,5-dimethylphenol

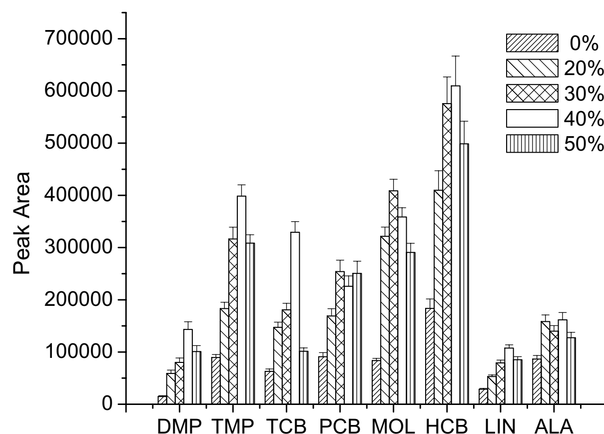

Fig. 4. Effect of acetone composition on LPME efficiency ($n = 3$). Final concentration of each analyte in spiked soil sample = 0.5 μg/g. See Fig. 1 for explanations of abbreviations.

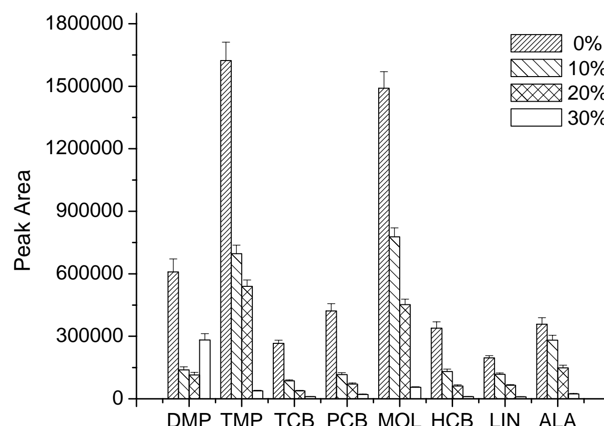

Fig. 6. Effect of NaCl concentration on LPME efficiency ($n = 3$). Final concentration of each analyte in spiked soil sample = 0.5 μg/g. See Fig. 1 for explanations of abbreviations.

Table 1
Quantitative results of LPME

Pesticides	Relative recovery (%)	Linear range (μg/g)	Linearity	% R.S.D. ($n = 6$)
2,5-Dimethylphenol	94	0.1–2	0.98	13
2,3,5-Trimethylphenol	100	0.1–2	0.98	6
1,2,4,5-Tetrachlorobenzene	99	0.2–2	0.99	7
Pentachlorobenzene	99	0.2–2	0.98	9
Molinate	99	0.2–2	0.98	6
Hexachlorobenzene	92	0.2–2	0.98	11
Lindane	99	0.2–1	0.99	6
Alachlor	97	0.2–1	0.99	9

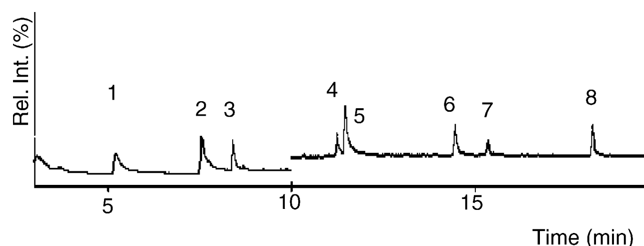

Fig. 7. GC–MS chromatogram of an aged soil sample extract after LPME. Peaks: (1) 2,5-dimethylphenol, (2) 2,3,5-trimethylphenol, (3) 1,2,4,5-tetrachlorobenzene, (4) pentachlorobenzene, (5) Molinate, (6) hexachlorobenzene, (7) Lindane, and (8) Alachlor. Conditions are given in the text. Abbreviation: rel. int., relative intensity.

(13%) and hexachlorobenzene (11%). The good precision may be due to the fast extraction, the use of a new fiber for every extraction and the direct GC–MS injection after extraction. The linearity of the method was tested over the range 0.1–2 μg/g of pesticides in soil. The LPME procedure showed a satisfactory linear behavior in the tested range, with correlation coefficients ranging between 0.98 and 0.99. As seen from Table 1, the relative recoveries (defined as the ratio of GC–MS peak areas of the respective experimental data to those calculated from the linearity equations for the extract at same spiked pesticide concentration) [17] studied at pesticide concentrations of 0.2 μg/g were in the range of 92–100%.

3.2.2. Precision and limits of detection (LOD)

Aqueous soil samples (0.5 μg/g) were studied by the developed LPME procedure, while SPME was also performed as comparison in terms of precision and limits of detection. The results are listed in Table 2. As seen from Table 2, SPME gave relatively poor precision, with R.S.D. ranging from 6–18% for 30 min extractions. The reason for this may be that the soil particles and possibly the salt in the aqueous soil sample adsorbed on the fiber and seriously affected the analysis. Also, the integrity of the fiber was compromised. In comparison, the precision of LPME in 4 min extraction ranged from 5–10%. It seemed that the present method was able to overcome the problems encountered in normal SPME because of the protection afforded by the porous hollow fiber. The small pore size allowed the hollow fiber to function as a clean-up filter that prevented large molecules and particles in the sample matrices from being extracted into the organic solvent. Also, a new hollow fiber was used for each extraction. This eliminated matrix and carry-over effects that could occur with SPME if the fiber was reused. The limits of detection, based on a signal-to-noise ratio of 3, ranged from 0.05 to 0.1 μg/g for LPME. SPME gave better LODs (0.01–0.06 μg/g) (30 min extraction) than LPME (only ~4 min). This is probably because the analytes were more efficiently extracted from the slurry by SPME than by LPME under the experimental conditions. Under the conditions explored, the analytes apparently were more efficiently extracted by SPME. However, the LPME technique developed has the advantage of a short extraction time (~4 min versus 30 min for SPME). With a 4 min extraction time, SPME would not achieve better LODs than LPME. Also, as mentioned before, the SPME precision is poorer and the fiber cannot be used satisfactory for multiple extractions. LPME is characterized by fast extraction, easy operation, affordability, and better precision.

Table 2
Comparison of extraction of pesticides from aqueous soil sample by LPME and SPME ($n = 4$)

Pesticides	Hollow fiber-protected LPME		SPME	
	% R.S.D.	LODs (μg/g)	% R.S.D.	LODs (μg/g)
2,5-Dimethylphenol	10	0.07	17	0.02
2,3,5-Trimethylphenol	5	0.05	6	0.01
1,2,4,5-Tetrachlorobenzene	6	0.1	9	0.05
Pentachlorobenzene	9	0.05	16	0.02
Molinate	5	0.08	7	0.02
Hexachlorobenzene	10	0.1	18	0.03
Lindane	6	0.09	8	0.06
Alachlor	9	0.09	16	0.05

3.3. Extraction from aged soil sample

The spiked (2 μg/g) soil sample was stored in a capped vial that was kept in a desiccator for 2 months. The final pesticide content of this soil was not known due to possible evaporative losses during preparation and prolonged storage. This aged soil resembled a real contaminated sample (more so than the common technique of spiking one spot in the soil matrix just before analysis), because the target analytes were in more intimate contact with the soil particles, thus maximizing analyte/matrix interaction [22]. The soil sample was subjected to LPME and GC–MS analysis. Fig. 7 shows a typical chromatogram of an extract. The standard addition method was used for quantification. The results are shown in Table 3. As seen, all the pesticides could be detected by LPME with good precision. The variation in recoveries observed may be due to the different evaporative, adsorptive,

Table 3
Extraction results of pesticides in aged soil sample by LPME ($n = 3$)[a]

Pesticides	% R.S.D.	Concentration (μg/g)	% Recovery
2,5-Dimethylphenol	11	1.4	70
2,3,5-Trimethylphenol	4	1.8	90
1,2,4,5-Tetrachlorobenzene	7	0.51	26
Pentachlorobenzene	9	0.34	17
Molinate	3	1.5	75
Hexachlorobenzene	10	0.13	7
Lindane	4	0.92	46
Alachlor	7	0.23	12

[a] Final concentration of analyte in spiked soil sample = 2 μg/g before storage.

and degradation characteristics of each analyte in the spiked soil sample during prolonged storage.

4. Conclusion

The proposed liquid-phase microextraction technique utilizes a simple and disposable extraction device. It requires very little sample solution (4 ml) and organic solvent (ca. 4 μl), respectively. By addition of acetone to the soil sample, we can detect pesticides from soil samples at low microgram per gram levels. The procedure has some limitations. It cannot yet be easily directly coupled to GC–MS. The extraction process itself can be automated by using a syringe pump but transfer of extract for analysis is still manually performed. Selection of a suitable solvent for particular classes of analytes is also not straightforward. Nevertheless, from the results of our experiments, liquid-phase microextraction combined with GC–MS has been demonstrated to be viable, easy to use, and rapid for analysis of pesticides in soil samples.

Acknowledgement

This work was financially supported by the National University of Singapore.

References

[1] H.B. Lee, L.D. Weng, A.S.Y. Chau, J. Assoc. Off. Anal. Chem. 67 (1984) 789.
[2] F. Navarro-Villoslada, L.V. Pérez-Arribas, M.E. León-González, L.M. Polo-Díez, Anal. Chim. Acta 308 (1995) 238.
[3] F. Hernandez, J. Beltran, F.J. Lopez, J.V. Gaspar, Anal. Chem. 72 (2000) 2313.
[4] M.N. Sarrión, F.J. Santos, M.T. Galceran, Rapid Commun. Mass Spectrom. 14 (2000) 2271.
[5] R-A. Doong, P-L. Liao, J. Chromatogr. A 918 (2001) 177.
[6] A. Bouaid, L. Ramos, M.J. Gonzalez, P. Fernández, C. Cámara, J. Chromatogr. A 939 (2001) 13.
[7] J. Beltran, F.J. Lopez, F. Hernandez, J. Chromatogr. A 885 (2000) 389.
[8] Z. Zhang, J. Poerschmann, J. Pawliszyn, Anal. Commun. 33 (1996) 219.
[9] M. de Fatima Apendurada, J. Chromatogr. A 889 (2000) 3.
[10] Y. Wang, Y.C. Kwok, Y. He, H.K. Lee, Anal. Chem. 70 (1998) 4610.
[11] M.H. Ma, F.F. Cantwell, Anal. Chem. 71 (1999) 388.
[12] L.S. de Jager, A.R.J. Andrews, J. Chromatogr. A 911 (2001) 97.
[13] E. Psillakis, N. Kalogerakis, J. Chromatogr. A 907 (2001) 211.
[14] K. Carlsson, B. Karlberg, Anal. Chim. Acta 415 (2000) 1.
[15] A.L. Theis, A.J. Waldack, S.M. Hansen, Anal. Chem. 73 (2001) 5651.
[16] L.M. Zhao, H.K. Lee, Anal. Chem. 74 (2002) 2486.
[17] G. Shen, H.K. Lee, Anal. Chem. 74 (2002) 648.
[18] H.Y. Zhang, A.R.J. Andrews, J. Environ. Monit. 2 (2000) 656.
[19] G. Shen, H.K. Lee, Anal. Chem. 75 (2003) 98.
[20] S. King, J.S. Meyer, A.R.J. Andrews, J. Chromatogr. A 982 (2002) 201.
[21] R. Baciocchi, M. Attinà, G. Lombardi, M.R. Boni, J. Chromatogr. A 911 (2001) 135.
[22] M. Llompart, K. Li, M. Fingas, Talanta 48 (1999) 451.