OPTIMISATION OF SOLID PHASE MICROEXTRACTION (SPME) CONDITIONS FOR HEADSPACE ANALYSIS OF ORGANOPHOSPHATE PESTICIDES IN WHOLE BLOOD

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ABSTRACT: Organophosphate (OP) pesticides are a significant cause of fatal poisoning in many countries. Problems exist in detecting the parent substances in autopsy blood when putrefaction has occurred. This study evaluated the use of SPME as an extraction technique for the analysis of OP pesticides in whole blood. Malathion and Diazinon were used as model compounds, added to whole blank blood. Parameters affecting the adsorption of analyte on the SPME fibre were sampling time, temperature, agitation, and modification of the ionic strength and pH. The best conditions for extraction were at 90°C, with a conditioning time of 10 minutes, exposure time of 30 minutes and desorption time of 5 minutes. All samples were agitated during the extraction process. The primary detection and quantification technique was GC-NPD but GC/MS was also used as a confirmatory identification technique. The minimum detectable level by GC-NPD was 100 ng per 0.5 ml whole blood and the linear quantifiable range was from 200–1000 ng per 0.5 ml whole blood (correlation coefficient 0.993). Recoveries were approximately 0.39% with a coefficient of variation of 6.8%. The rates of *in vitro* change of Malathion concentrations in blood during storage at ambient temperatures in the range 20–25°C and at 4°C were also measured. The time limit of detection at 20–25°C was 4 h but at 4°C was more than 11 days. No interferences from the putrefying blood were observed. From the results it was concluded that headspace-SPME could be used as an alternative technique for sample preparation which is simpler and more rapid than liquid-liquid extraction or conventional solid phase extraction.

KEY WORDS: Solid phase microextraction; Organophosphates pesticides; GC/MS.

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INTRODUCTION

In 1990, SPME was introduced by Arthur and Pawliszyn [1]. This method is a new, fast, and simple analytical technique which employs a stationary phase of polydimethylsiloxane coated on a fused-silica fibre to extract analytes from aqueous or gaseous samples in sealed vials using direct immersion or headspace techniques

respectively. The subsequent analyses are currently performed by GC and HPLC and the analytes are desorbed in the injector of the gas chromatograph. This method represents a further advance as a solvent-free alternative to the extraction of organic compounds from biological samples [2, 9]. Headspace-SPME had been considered suitable only for extraction of volatiles but the results for tricyclic antidepressants [4] and local anaesthetics [3] seem to open the applicability of headspace-SPME to a number of other solid drugs and poisons of medium-sized molecular weights.

More recently headspace-SPME of biological fluids was applied to analyses such as cyanide in human whole blood [8], and organophosphates and carbamates in blood and urine [5, 7].

SPME is a process dependent on equilibrium rather than total extraction: the amount of analyte extracted at a given time is dependent on the mass transfer of an analyte through the aqueous phase. The principle behind SPME is the equilibrium partition process of the analyte between the fibre coating and the aqueous solution (including the gaseous phase for headspace analysis). The time to equilibrium is a function of the analyte and conditions used (e.g. fibre chemistry and thickness).

We will look at these factors by focussing on the analyses of Malathion and Diazinon (as internal standard) in blood.

EXPERIMENTAL

Materials

Malathion and Diazinon were purchased from Promochem Limited, UK. Stock solutions containing 100 mg per ml were prepared in methanol. Sodium chloride and hydrochloric acid (HCl) were AR Grade and were used to prepare saturated sodium chloride and 0.1 M HCl solutions.

Headspace vials (4.0 ml amber, screw top) with septum caps (PTFE/silicone septa) were heated and agitated with a Corning hot plate stirrer. SPME sampling stands (holding 8 vials) and magnetic stirrer (10 x 3 mm covered with PTFE) were used. A manual assembly for SPME, with replaceable 100 mm extraction fibre coated with polydimethylsiloxane, was obtained from Supelco Sigma-Aldrich Company Ltd, UK.

Instrumentation

Gas chromatography used a Hewlett-Packard Model 5890 Series II. Gas chromatograph equipped with a nitrogen-phosphorus detector, a 0.75 mm splitless insert and an HP1 capillary column (30 m x 0.53 mm i.d., 0.88 mm phase thickness). The column oven was programmed from an initial temperature of 100°C (held for 2 minutes) to 300°C at a ramp rate of 10°C per minute. Injector and detector temperatures were 250°C and 280°C respectively.

Headspace-SPME procedure

A polydimethylsiloxane (PDMS) coated fibre (diameter 100 mm) was exposed to a temperature of 250°C in the GC injection port overnight prior to the analyses. This removed contaminants as well as conditioning the fibre.

DETERMINATION OF EXTRACTION PROCEDURE

Optimisation of desorption conditions

The SPME fibre, exposed to 1.0 ml blood containing Malathion and Diazinon at a concentration of 10 ppm, was inserted into the GC inlet and left for various time intervals (1–5 minutes). A check was made for carry over or traces of peaks of no interest.

Effect of temperature

A set of 4.0 ml vials was prepared with magnetic stirrer and 1.0 ml of blood containing a concentration of 10 ppm Malathion and Diazinon. The vials were place in a vial receptacle on a hot plate/stirrer at 80°C and maximum speed. After heating for 10 minutes, the septum-piercing needle of the SPME was passed through the septum (Figure 1). The pre-treated fibre was pushed out from the needle and exposed in the headspace for 5, 10, 15, 20, 30, 40, 50 and 70 minutes to allow the adsorption of the compound. The fibre was withdrawn into the needle and pulled out from the vial. It was then inserted into the injection port of the gas chromatograph; the fibre was exposed for 5 minutes to ensure complete desorption of the compound. The same procedure was applied to a second set of samples at an incubation temperature of 90°C.

Effect of agitation

Two sets of blood samples were prepared as in described above and placed in the heating block at 80°C. One set was agitated and the other not. Time extraction profiles were established from 0–70 minutes.

Additives and pH adjustment

Three sets of 6 vials were prepared containing 0.5 ml blood spiked with 1000 ng of Malathion and Diazinon. The first set was diluted with 0.5 ml distilled water. The second set was diluted with 0.5 ml 0.1 M HCl. The third set was diluted with 0.5 ml water, 100 ml 0.1 M HCl and 100 ml saturated sodium chloride. All three sets were treated as above (see “Effect of temperature”) but at a sample temperature of 90°C.

The results of the work described in chapter “Determination of extraction procedure” formed the basis for optimal conditions for subsequent studies.

Fig. 1. Schemtic ilustration of the headspace-SPME method.

Limit of detection (LOD)

Aliquots of blood (0.5 ml) containing Malathion at concentrations of 500 ng, 300 ng, 100 ng and 80 ng Malathion per 0.5 ml blood were analysed in triplicate with the developed procedure. Blank injections were performed between each run to ensure carry over, if present, was negligible.

Reproducibility

The analysis was repeated ten times at Malathion concentrations of 200 ng and 800 ng per 0.5 ml whole blood using the optimised conditions.

Linearity/calibration curves

Calibration standards with Malathion concentrations in the range 200 ng to 1000 ng per 0.5 ml whole blood were prepared. Internal standard Diazinon was added at an amount of 60 ng per vial. The precision of the method at 200 ng and 800 ng/0.5 ml was measured by analysing 10 samples at each concentration.

Stability of Malathion during storage in a fridge (0–4°C) and at ambient temperature (20–25°C).

Fresh blood (20 ml) was spiked with Malathion to a concentration of 10 ppm. The sample was split into 2 halves. One half was kept in the fridge (0–4°C) and the other was exposed to a room temperature of 20–25°C. The specimens were analysed every 30 minutes for the first 8 hours and then each day for 11 days.

RESULTS

Effect of agitation and temperature

The equilibrium state was achieved faster for Malathion at the higher temperature but the amount extracted was lower compared to that at the lower temperature. This due to the effect of the temperature on the fibre. At higher temperatures the diffusion coefficient in blood is higher and the extraction time is shorter, but the partition coefficient in the fibre is also lower. For Diazinon, the higher the temperature the better the response and equilibrium was also attained faster at the higher temperature.

Salting effect and pH adjustment

Two other common techniques to enhance extraction are addition of salt and pH adjustment. From Figures 7–10, the results indicated that both of these influenced the amount extracted.

By addition of salt and pH adjustment the equilibrium state can be attained much more rapidly. If only acid is used, the amount extracted is higher in the case of Malathion but

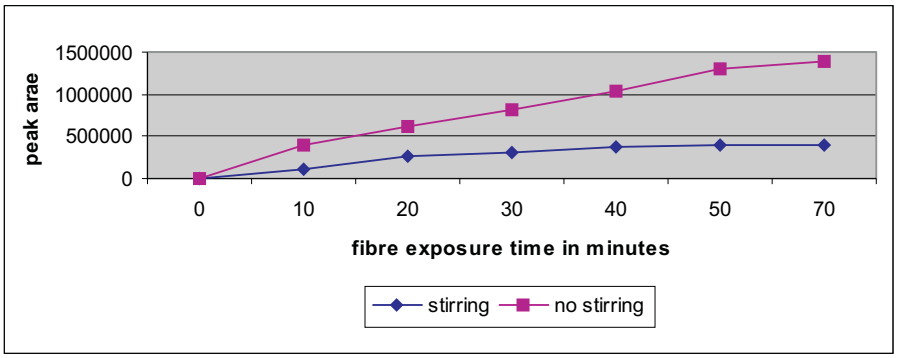

Fig. 2. Extracted blood with Malathion and Diazinon.

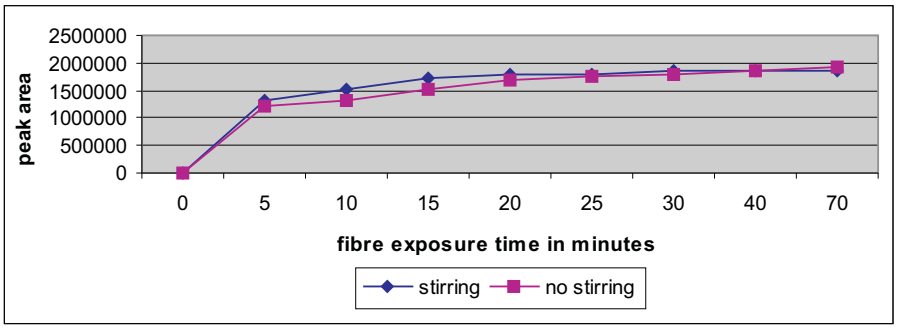

Fig. 3. Effect of stirring on analysis of Diazinon.

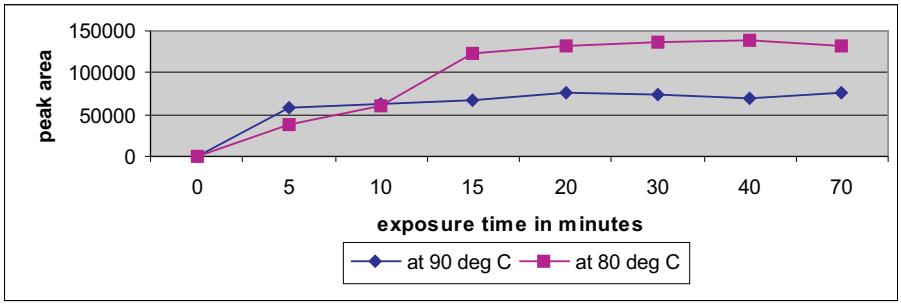

Fig. 4. Extraction time profile for Malathion in blood.

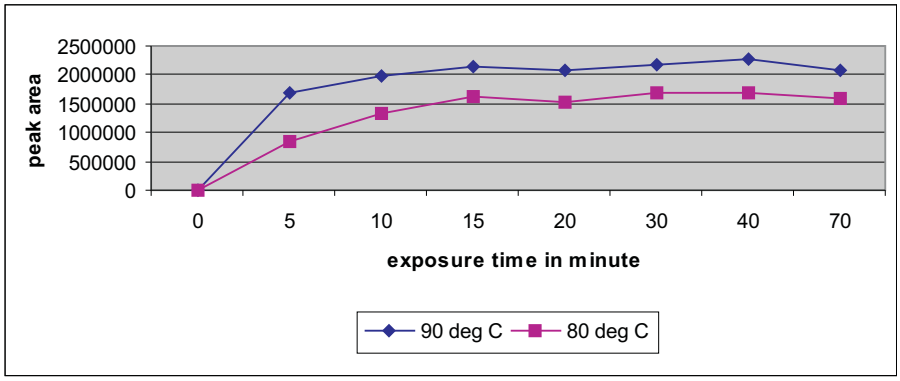

Fig. 5. Extraction time profile for Diazinon.

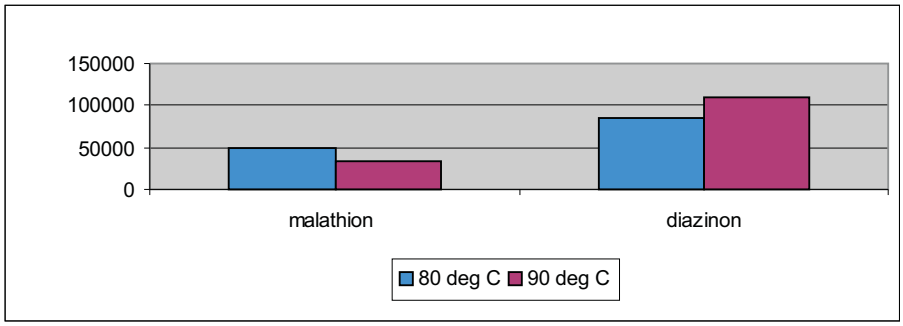

Fig. 6. Extraction at 80 and 90°C.

equilibrium is not achieved even after 60 minutes fibre exposure, whereas after diluting with water the equilibrium was achieved faster but the amount extracted was lower compared to that of salting and acidification. Acidification reduces the response for Diazinon. Diluting the blood also had a significant impact on the amount extracted.

Based on the results given in "Effect of agitation and temperature" and "Salting effect an adjustment", the developed extraction method is as follows: a blood sample

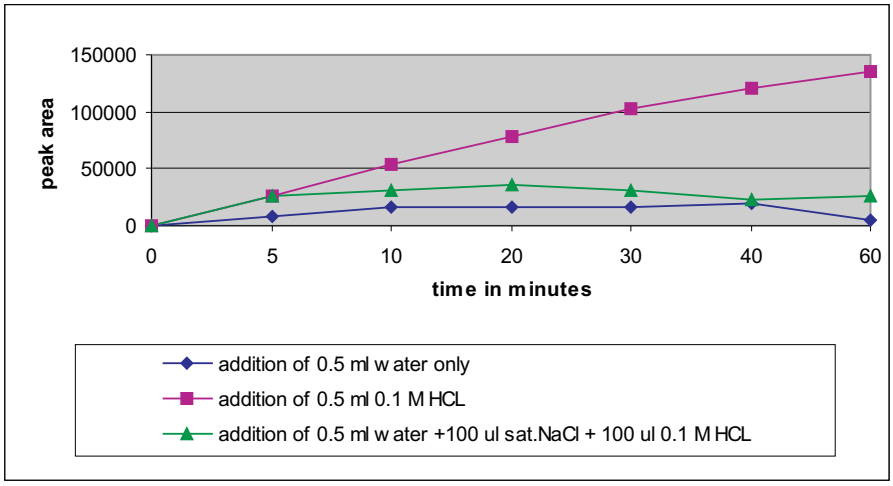

Fig. 7. Extraction time profile of Malathion after acid and salt addition.

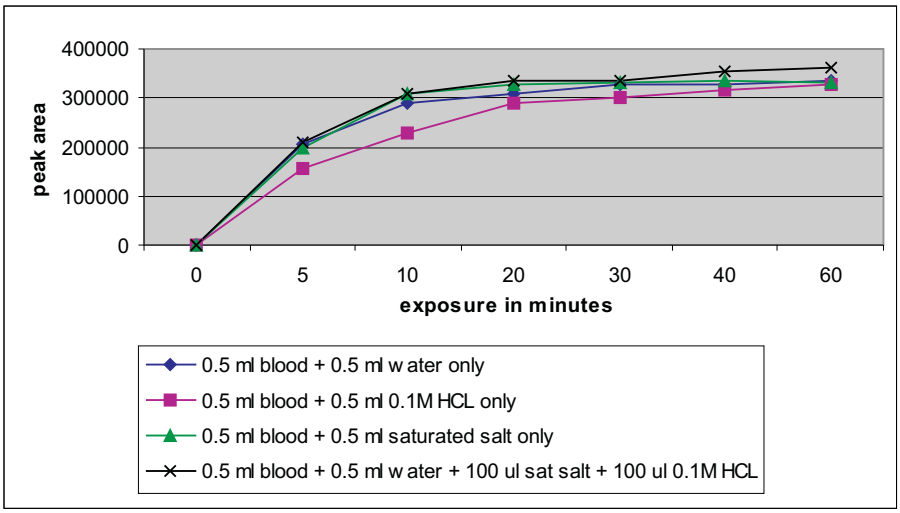

Fig. 8. Extraction time profile of Diazinon after acid and salt addition.

(volume 0.5 ml) is diluted with 0.5 ml distilled water followed by addition of 100 ml of 0.1 M HCl and 100 ml saturated sodium chloride solution. Extraction consists of 10 minutes sample equilibration and 30 minutes fibre exposure to the sample headspace. Desorption time in the GC inlet is 5 minutes.

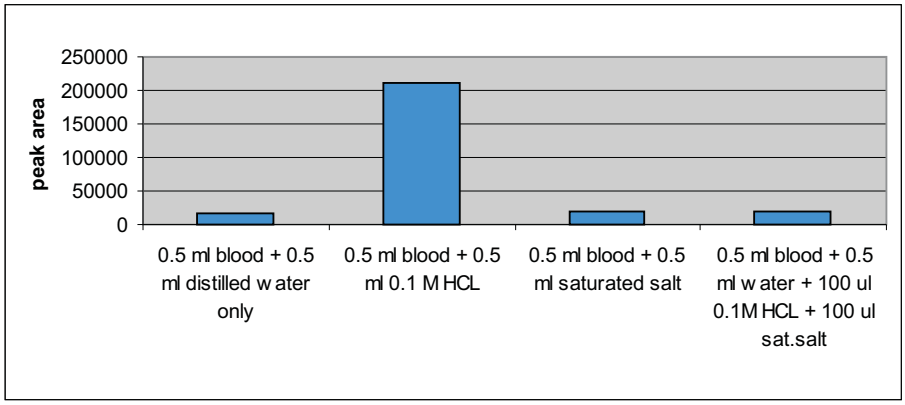

Fig. 9. Malathion extracted after acid and salt addition.

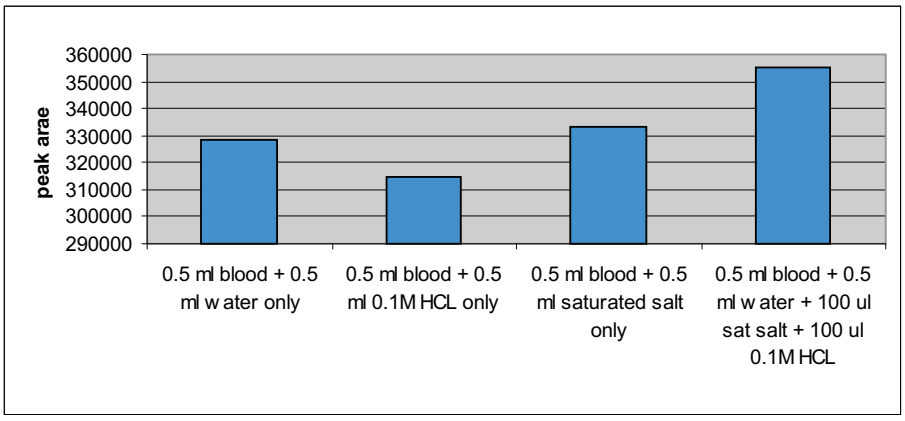

Fig. 10. Diazinon extracted after acid and salt addition.

Detection limit, reproducibility study, quantifiable level and linearity

The limit of detection was found to be 100 ng per 0.5 ml blood. Retention times for Malathion and Diazinon (internal standard) were 15.9 minutes and 14.1 minutes, respectively. Recovery at 200 ng per 0.5 ml blood was 0.39% and coefficient of variation 6.8% whereas at 800 ng per 0.5 ml blood was 0.38% and coefficient of variation 6.5% (n = 5). The method was linear in the range 200 ng to 1000 ng per 0.5 ml blood (n = 3) and the correlation coefficient (r^2) was 0.993. At concentrations of 200 ng and 800 ng/0.5 ml blood, the method was found to have a coefficient of variation of 4.40% and 8.14%, respectively.

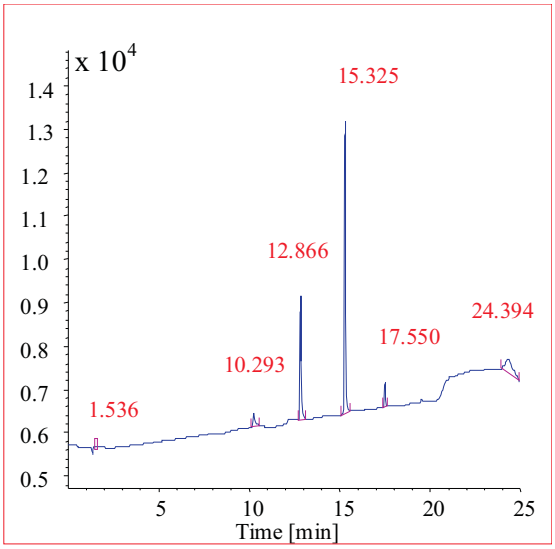

Fig. 11. Blank blood.

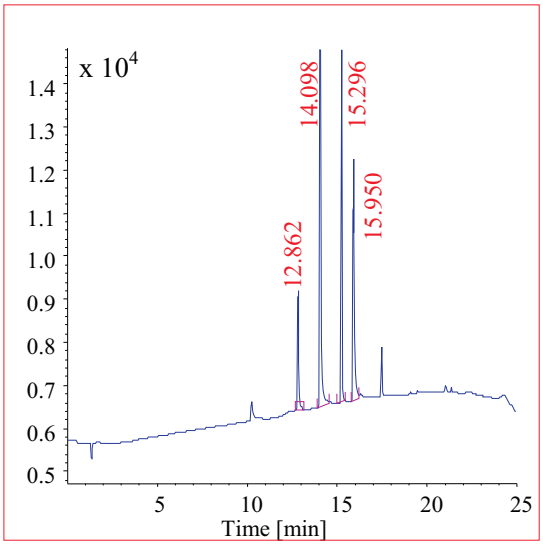

Fig. 12. Extracted blood with Malathion and Diazinon.

Stability of Malathion in whole blood

Malathion disappeared (not detected) from fresh blood after about 4 hours at a room temperature of 20–25°C (Figure 9). The half-life ($t_{1/2}$) was found to be 1.1 hours at pH 6–7. Malathion was found to be stable if the sample was kept under cold conditions without much deviation (concentration of 10 ppm) but the measured concentration still dropped initially.

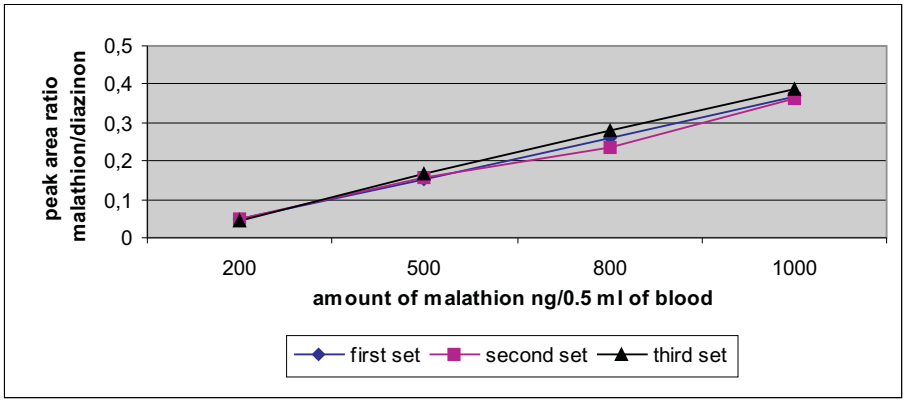

Fig. 13. Calibration curve for Malathion at 90°C.

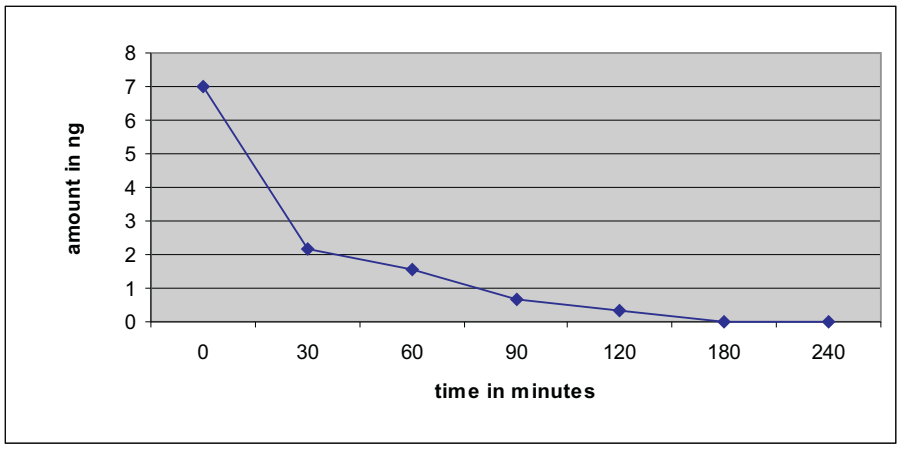

Fig. 14. Rate of Malathion disappearance at 20–25°C.

DISCUSSION

Since Malathion and Diazinon each have a low Henry's constant (semi-volatile pesticide), the sample is heated in order to increase their concentrations in the gas phase in headspace-SPME. Although heating is often useful to enhance sensitivity it has a double impact: at higher temperature, diffusion coefficients in blood are higher and the extraction time is shorter but the net partition coefficients are lower. The difference arises from the fact that, with SPME, there are three phases and heating alters the partitioning of analyte between the headspace and the fibre to favour the headspace. Two

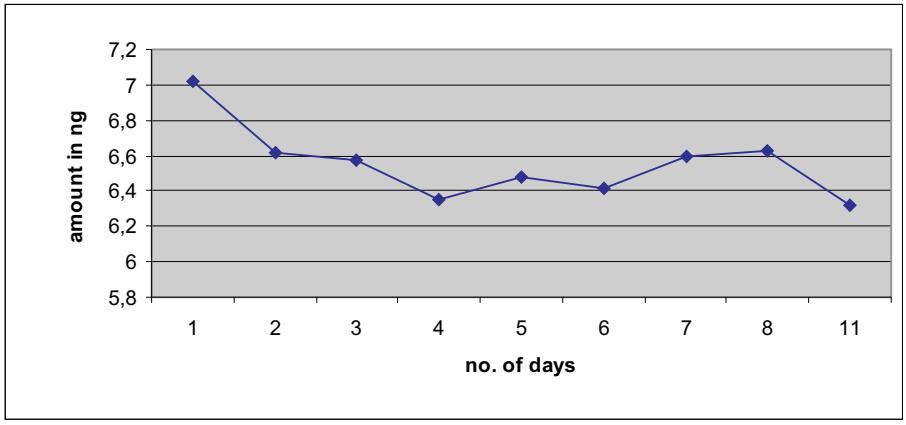

Fig. 15. Stability of Malathion in blood at 0–4°C.

temperature levels were evaluated, i.e. at 80° and 90°C. At the higher temperature the amount of Malathion extracted was reduced (Figure 4) due to a reduction in the distribution constant in the fibre but examination of the extraction time profile showed that equilibrium was attained faster at the higher temperature.

Agitation improved the equilibrium attained and the time required for Malathion (Figure 2 and Figure 3) though these were not so marked for Diazinon. Also, modification of the ionic strength and the pH had an impact on the sorption (affinity) of the analyte for the fibre coating and equilibrium was achieved much faster and with a higher recovery. Reducing the solubility of the analyte in the aqueous phase can increase the amount of the analyte extracted by the fibre. This can be achieved by the addition of salt and/or by pH adjustment. It is important to remember that in SPME neither complete extraction of analytes nor full equilibrium is necessary [6], but consistent sampling time, temperature, fibre immersion depth and headspace volume are crucial to reproducibility. However, extraction at nonequilibrium results in a lower degree of reproducibility of the analysis.

CONCLUSION

The technique was applied successfully to study the rate of disappearance of Malathion from whole blood. The technique is simple but careful consideration has to be taken into account. Advantages of SPME are that not much preparative work needs to be done and the use of solvent is eliminated, thus reducing pollution and health hazards. Also, only a small sample volume is needed for extraction. The major drawbacks are the fragility of the fibre and time consuming nature of the technique if done manually.

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