Original

Direct Analysis of Permethrins in Human Blood by SPE–GC/MS

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Received for review June 25, 2007, Accepted September 28, 2007.

Abstract

A direct method to determine permethrins in human whole blood with high recovery and high sensitivity was developed using a combination of solid–phase extraction (SPE) and gas chromatography–mass spectrometry (GC/MS). Whole blood was diluted with pure water and then loaded on a preconditioned solid–phase Oasis HLB cartridge ; permethrins were eluted by toluene. The extract was mixed with an internal standard and then concentrated under a N_2 gas stream before being analyzed by a capillary GC/MS in electron ionization mode. Compounds of *cis*– and *trans*–permethrin were separated and quantified in selected ion monitoring mode. The SPE recovery efficiency of *cis*– and *trans*–permethrin was 97.7 ± 7.9% and 99.7 ± 7.7%, respectively. The LOD and LOQ found for *cis*–permethrin and *trans*–permethrin in whole blood were 0.21 and 0.67 ng/ml, and 0.20 and 0.64 ng/ml, respectively. Using this method, we measured permethrins in the blood of pest control operators.

Keywords : gas chromatography–mass spectrometry, *cis*–permethrin, *trans*–permethrin, solid–phase extraction, whole blood, pest control operator

1. INTRODUCTION

Permethrins have been widely utilized worldwide as a synthetic pyrethroid insecticide in agriculture, forestry, home pest control, public health and in similar contexts [1]. It is well known that the toxicity of permethrins is low in mammals, including humans [1, 2]. As well all synthetic pyrethroids, there have been reports of permethrins–related neurotoxicity and skin paresthesia [2–4].

Biologically, permethrins are rapidly hydrolyzed at the central ester bond in the chemical structure and are oxidized to detoxified–metabolites, 3–(2,2–dichlorovinyl)–2,2–dimethylcyclopropane–carboxylic acid (Cl_2CA) and 3–phenoxybenzoic acid (3–PBA). These metabolites are renally eliminated with a half–life of about 6 hr [5]. Many analytical methods for Cl_2CA and 3–PBA have been developed using LC–tandem MS or GC/MS because these compounds are useful for biological monitoring of permethrin exposure [6, 7]. In the presence of an esterase inhibitor, permethrin detoxification is blocked or low esterase activity causes delayed detoxification. In such cases, permethrins accumulate in the human body. Af-

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ter the recent Gulf War (1990), it has been reported that permethrin has complex effects in the presence of other chemicals and that these enhance the toxicity of permethrin itself [8]. Measurement of permethrins as well as Cl_2CA and 3–PBA present in the blood and urine of permethrin–exposed humans is required for the biological study of permethrins.

There are reports that have analyzed permethrins as pesticides in human blood but most of these have been for serum or plasma samples, and not for whole blood [9–12]. The analytical results for serum or plasma samples may not account for permethrins bound to cell membranes in blood because many pesticides, including permethrins, are lipophilic and bind to the cell membrane of erythrocytes [13]. Thus, a method for total assay of permethrins in whole blood is necessary. A study has shown that the determination of synthetic pyrethroid insecticides, including permethrins in whole human blood, has a high recovery rate using liquid–liquid extraction [14]. Recently, SPE methods have been developed for analyses of environmental, food, and physiological samples to allow simplification of the elaborate processes of detection required in the laboratory. As well known, SPE is simple and effective for cleaning up biological samples prior to quantitation. The recovery of permethrins has been reported to be about 85% for SPE with a C_{18} cartridge of human plasma [11, 12]. However, there have been no reports of the use of SPE for the determination of permethrins in whole blood.

This study aimed to develop a method for detecting and quantifying permethrins in whole blood with high rates of recovery and high sensitivity using a recent SPE sorbent and GC/MS. The GC/MS enabled separation and quantification of two isomeric permethrins, the *cis*– and *trans*–forms ; this is important because cis–permethrin toxicity is known to be higher because of its slow detoxification [1, 2]. Using the method we developed, we then determined the permethrin concentration in whole blood from pest control operators who were occupationally exposed to insecticides.

2. EXPERIMENTAL

2.1. Chemicals

The permethrin mixture (1 mg/ml in toluene, *cis*– and *trans*–permethrin mixture) was purchased from NANOGENS Inc. (Watsonville, CA, U.S.A.). The standard solutions, 10 μg/ml of *cis*–permethrin and *trans*–permethrin in cyclohexane were purchased from Sigma–Aldrich Co. (St. Louis, MO, U.S.A.). The 10 μg/ml of deuterated–fenitrothion (fenitrothion–d_6) in acetone was purchased from Hayashi Pure Chemical Ind. (Osaka, Japan). An Oasis HLB 6 cc (500 mg) LP Extraction Cartridge was purchased from Waters Co. (Milford, MA, U.S.A.). Ethanol, methanol, acetone and toluene of HPLC grade and n–hexane for residual material test purpose were purchased from Wako Pure Chemical (Osaka, Japan).

2.2. Working standard solutions

One hundred μg/ml of permethrins* in ethanol was prepared as a working standard solution from NANOGENS permethrin mixture, and was standardized with the Sigma–Aldrich permethrin solutions. One μg/ml of fenitorothion–d_6 was prepared as an internal standard for the GC/MS measurements. Each isomer was identified by the scan spectra obtained with electron ionization (EI) and the retention time. A series of permethrin solutions including a known amount of fenitorothion–d_6 (IS) were prepared by dilution for the GC/MS linearity test and for calculation of the limit of determination (LOD) and limit of quantitation (LOQ).

2.3. Blood samples

For the method development, human blood control samples were obtained from 2 healthy volunteers who were not exposed to permethrins, and were immediately treated with heparin and stored at −20°C ; they were used in a week. Blood samples from seven male pest control operators aged 25–59 years were also immediately treated with heparin, and stored at −80°C prior to analyses.

2.4. SPE method for permethrins in blood samples

The Oasis HLB cartridge used contained a copolymer (m–divinylbenzene/N–vinylpyrolidone) packing material (nominal 60 μm) designed to have hydrophilic–lipophilic properties, in the format of diameter of ca.12 mm x 14 mm long. Preconditioning of the SPE cartridge was performed with elution solvent to be used, according to the manufacture's instruction [15].

(1) Preconditioning

Before use, an Oasis HLB cartridge was washed with 5 ml of methanol and 5 ml of toluene in this order. Subsequently, 10 ml of methanol and 5 ml of Milli–Q water were passed through the cartridge before the sample solution was loaded.

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*This was a nominal concentration ; the exact values for each level of solution were as follows throughout this study :

1 ng/ml : 0.64 ng *cis*–permethrin and 0.31 ng *trans*–permethrin in ml ; total permethrin 0.95 ng/ml (*cis*/*trans*=67.7/32.3),

2 ng/ml : 1.28 ng *cis*–permethrin and 0.61 ng *trans*–permethrin in ml ; total permethrin 1.89 ng/ml (*cis*/*trans*=67.7/32.3),

5 ng/ml : 3.2 ng *cis*–permethrin and 1.5 ng t*rans*–permethrin in ml ; a total permethrin 4.7 ng/ml (*cis*/*trans*=67.7/32.3),

10 ng/ml : 6.4 ng *cis*–permethrin and 3.1 ng *tran*s–permethrin in ml ; a total permethrin 9.5 ng/ml (*cis*/*trans*=67.7/32.3),

20 ng/ml : 12.8 ng *cis*–permethrin and 6.1 ng *trans*–permethrin in ml ; total permethrin 18.9 ng/ml (*cis*/*trans*=67.7/32.3),

50 ng/ml : 32 ng *cis*–permethrin and 15 ng *trans*–permethrin in ml ; total permethrin 47 ng/ml (*cis*/*trans*=67.7/32.3), and

100 ng/ml : 64 ng *cis*–permethrin and 31 ng *trans*–permethrin in ml ; a total permethrin 95 ng/ml (*cis*/*trans*=67.7/32.3).

(2) Elution

Five ml of 5–times diluted blood sample was loaded on the preconditioned cartridge. The cartridge was washed with 5 ml of Milli–Q water and 5 ml of methanol, and then centrifuged at 3000 rpm for 10 min at 4°C to minimize residual water and methanol. As elution solvent, 2.5 ml of toluene was loaded on to this cartridge being placed in a test tube. Unless otherwise stated, this cartridge was left for 60 min at room temperature, and then the cartridge in the test tube was centrifuged at 3000 rpm for 10 min at 4°C and the extract was obtained.

(3) Concentration

The extract was added with a known amount of IS solution, and was concentrated under a stream of nitrogen gas. Finally, the volume of the extract was made up to 0.5 ml by adding toluene.

2.5. GC/MS measurements

All GC/MS measurements were performed with a GCMS–QP 5050A gas chromatograph–mass spectrometer equipped with an AOC–20S autosampler (Shimadzu Co., Kyoto, Japan). Separation column was DB–1, 100% polydimethylsiloxane capillary column, 30 m long with an internal diameter of 0.25 mm and film thickness of 0.25 μm (Agilent Technologies Inc., Palo Alto, CA, U.S.A.). Three μl was injected in splitless mode ; split valve was opened 2 min after injection. The injector temperature was set to 250°C. Helium was used as the carrier gas (inlet pressure : 117 kPa). The column temperature was initially held at 50°C for 2 min, and programmed to 200°C at a rate of 20°C/min, and was held at 200°C for 1 min, finally programmed to 290°C at a rate of 7°C/min and held for 5 min. The MS interface temperature was set at 250°C. The electron multiplier of MS was set to 1.20 kV. The dwell time for selective ion monitoring (SIM) was 200 ms for each ion. Permethrin was detected at ionization potential of 70 eV using target ions of permethrin fragments (*m/z* 163, 165, 183) and *m/z* 183, major ion of permethrins was used for the determination. The peak area ratio of permethrin (*m/z* 183) and fenitrothion–d_6 (*m/z* 283) was used for quantification.

2.6. Assay validation

2.6.1. Calibration curve

Seven–point calibration plots were made with SPE–processed control blood samples which were spiked with 1, 2, 5, 10, 20, 50 and 100 ng/ml of permethrin ; three replicates of the SPE was performed at each concentration level. The peak area ratios of permethrin (*m/z* 183) to IS (*m/z* 283) were used for the calibration curves.

2.6.2. Limits of detection and of quantitation

The LOD and LOQ of permethrin standard toluene solution and of blood permethrin extracted using the SPE were calculated based on 3.14 times the standard deviation (SD) and 10 times the SD, respectively, from the results of the 7 repeated GC/MS runs using 2.5 μg/ml total permethrin [16].

3. RESULTS AND DISCUSSION

3.1. Separation and detection of permethrin isomers

Figure 1 shows SIM chromatograms for permethrin–toluene solution of 10 ng/ml (A) and 2 ng/ml (B), respectively. As described in *Experimental*, the isomeric identification was made by comparing the retention time ; *cis*–permethrin was identified at a retention time of 20.86 min (peak 2) and trans–permethrin at 21.03 min (peak 3). The spectra for these were very similar and could not have been identified by mass spectrum. Multiple ion detection of permethrin (*m/z* 163, 165, 183) was useful for confirming permethrins ; the major fragment ion at *m/z* 183 was suitable for the determination.

3.2. SPE optimization

To quantitatively recover permethrin from blood sample, SPE operations with an Oasis HLB cartridge were examined. The elution solvents tested were methanol, acetonitrile and toluene. Table

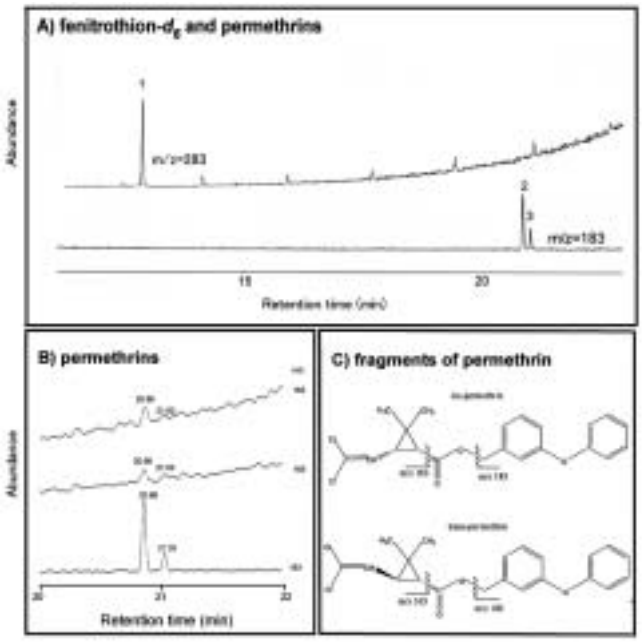

Figure 1. SIM mass chromatograms of permethrin and fenitrothion–d_6 (internal standard) in an ethanol solution. (A) The peak (1) at 12.75 min is for 100 ng/ml of fenitrothion–d_6, peak (2) at retention time 20.85 min is for the cis isomer of permethrin and peak (3) at 21.03 min is for the *trans*–isomer of permethrin (10 ng/ml of permethrin in total). (B)Multiple ions for 2 ng/ml total permethrin (*m/z* 163, 165, 183). (C) Structure of permethrins and the fragmentation.

Table 1. "Initial" recovery with solvents used for extraction of permethrins

	Recoveries of spiked permethrins (%, mean ± RSD)	
	cis	trans
Methanol (n=5)*	2 ± 1	1 ± 1
Acetonitrile (n=5)	16 ± 3	13 ± 2
Toluene (n=4)	85 ± 14	100 ± 28

* ; number of replicates

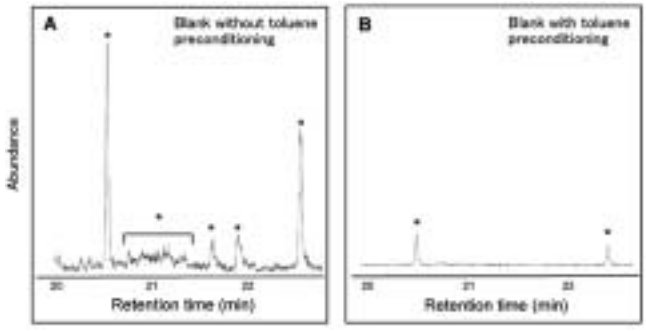

Figure 2. SIM mass chromatograms (*m/z* 183) of toluene extract blanks for the SPE cartridge.
(A) Blank for the SPE cartridge preconditioned without toluene, and (B) with toluene. Full scale is normalized to the same signal counts (2,000).
* ; specific blank peaks from Oasis HLB cartridge.

1 shows the "initial" recovery obtained with a standby time of zero before the centrifugation for a 100 ng/ml permethrin solution and 5 ml of each elution solvent. The highest permethrin recovery among those solvents tested was seen with toluene. Methanol did not help with permethrin recovery and the recovery was also limited with acetonitrile. Neither methanol nor acetonitrile was strong enough to extract permethrins from the concerned solid phase, whereas toluene efficiently extracts permethrins from it since toluene strongly interacts with poly(divinylbenzene/vinyl pyrolidone) base of the sorbent and separates permethrins from other lipophilic compounds. Therefore, toluene was of a choice as eluting solvent in this study. The elution volume was examined using 2.5 or 5.0 ml of toluene. Total permethrins recovery by extraction with 5.0 ml of toluene was 100 ± 8.7% ; recovery with 2.5 ml of toluene was 110 ±7.1% (n=3). The volume of toluene was therefore fixed at 2.5 ml throughout the study. Before elution with toluene, methanol was passed through the cartridge to minimize residual water which is less miscible with toluene and also to remove the unwanted methanol soluble constituents of blood.

Figure 2 shows the elution from Oasis HLB cartridges with toluene, when a cartridge was preconditioned without toluene (A) and with toluene (B). The first shows the higher background on the

Table 2. SPE recoveries of permethrins spiked into diluted blood and physiological saline

	Recoveries of spiked permethrins (%, mean ± RSD)	
	cis	trans
Whole blood (n=5)*	97.7 ± 7.9	99.7 ± 7.7
Physiological saline (n=8)	105.6 ± 12.1	102.7 ± 14.1

* ; number of replicates

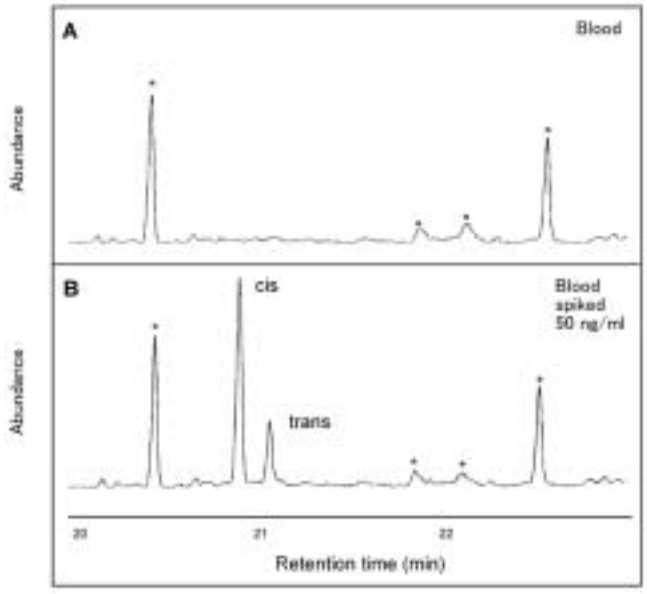

Figure 3. SIM mass chromatograms (*m/z* 183) of the extract from the SPE cartridge loaded with permethrin–spiked blood.
(A) Blank blood and (B) permethrin–spiked blood (50 ng/ml of permethrin).
Full scale is normalized to 75,000 signal counts.
* ; specific blank peaks from Oasis HLB cartridge.

chromatograms and the detection of permethrins was more difficult as these could not be easily distinguished from the background. When the cartridge was preconditioned with toluene, as seen in Fig. 2 B, the baseline quality was improved. Some specific peaks marked with (*) were identified as homologs of hydrocarbons from the GC/MS scan measurements. The chromatograms of the background of the cartridge and of the control blood sample spiked with permethrin are shown in Fig. 3. There was no interference between the specific peaks and both the *cis*– and *trans*–permethrin peaks.

As described in *Experimental* section, the blood samples were diluted with water before the SPE. To examine the recovery variation with the medium, a physiological saline which is characterized with pH of 7.4 and ionic strength by 0.85% NaCl, was spiked with permethrin. Table 2 shows the recovery rate of permethrin spiked in water–diluted blood and in physiological saline. The re-

covery of permethrin in water–diluted blood and the recovery of permethrin spiked in physiological saline were both close to 100%. This suggests that permethrins are recovered sufficiently from the cartridge. In contrast to the present study, the recovery of permethrins in plasma using a Sep–Pak C_{18} (Waters) cartridge was reported to be 81–93% [11] ; the present SPE with the Oasis HLB cartridge therefore performs better for permethrin recovery.

A comparative extraction of permethrins from whole blood was made using liquid–liquid extraction [14] ; we found the same recovery level as for our SPE method in that the recovery rates of *cis*– and *trans*–permethrin by hexane/acetone=80/20(v/v) were 105.3 ± 6.9% and 106.6 ± 6.7%, respectively. These results show that this SPE using an Oasis HLB cartridge is suitable for extracting permethrins from a whole blood sample ; moreover, the method has a high recovery rate and is ease to use. The present SPE method requires that blood be diluted only as part of analytical preprocessing ; this may potentially be extendable to the analyses of other lipophilic substances in blood.

3.3 Quantitative assay of blood permethrins

3.3.1. Calibration curves for the spiked blood samples

The separate quantification of *cis*– and *trans*–permethrin is required for biological monitoring because permethrin toxicity is dependent on the isomeric form and also because the metabolic rates of these isomers are different in body [2]. The proportion of the two isomers differs among commercial insecticides. However, there are few studies in which each the permethrin isomers were separated for quantification. In our study, calibration curves for *cis*– and *trans*–permethrin were obtained between 0.64 and 32 ng/ml, and between 0.31 and 31 ng/ml, respectively, as seen in Fig. 4. Figure 4 shows good correlation for each isomer dissolved in toluene and in permethrin–spiked control blood.

To evaluate LOD and LOQ, 1.6 ng/ml of *cis*–permethrin and 0.90 ng/ml of *trans*–permethrin in blood were assayed 7 times. The LOD of *cis*– and *trans*– permethrin in permethrin–spiked whole blood was calculated as 0.21 ng/ml (1.3 pg) and 0.20 ng/ml (1.2 pg), respectively. The LOQ of blood permethrin was 0.67 ng/ml (4.0 pg) for *cis*–permethrin and 0.64 ng/ml (3.8 pg) for the trans isomer. The present analysis is superior to previously reported methods, which have given LOD and LOQ values of 0.5 ng/ml and 1.0 ng/ml from plasma, respectively [12,17].

3.3.2. Permethrin detection in whole blood samples from pest control operators

The developed method was then used for a practical blood sample analysis. From one blood sample, permethrins exceeded the LOQ was detected as shown in Fig. 5 with 1.8 ng/ml of total permethrin consisting of 0.78 ng/ml *cis*–permethrin and 1.1 ng/ml

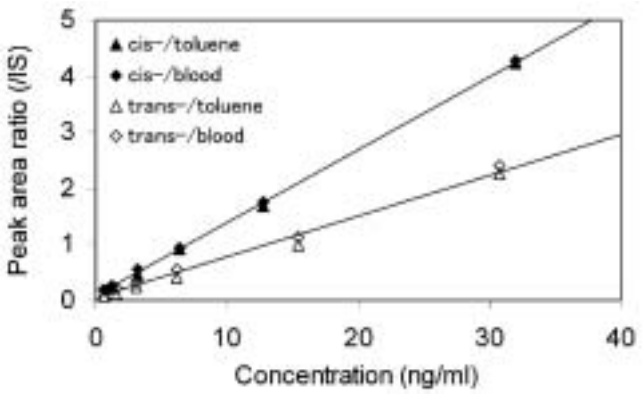

Figure 4. Linear calibration for *cis*– and *trans*–permethrin in toluene and in blood spiked with various levels of permethrin.
The caption, *cis*–/toluene indicates *cis*–permethrin in toluene ; similar notation applies for other combination.

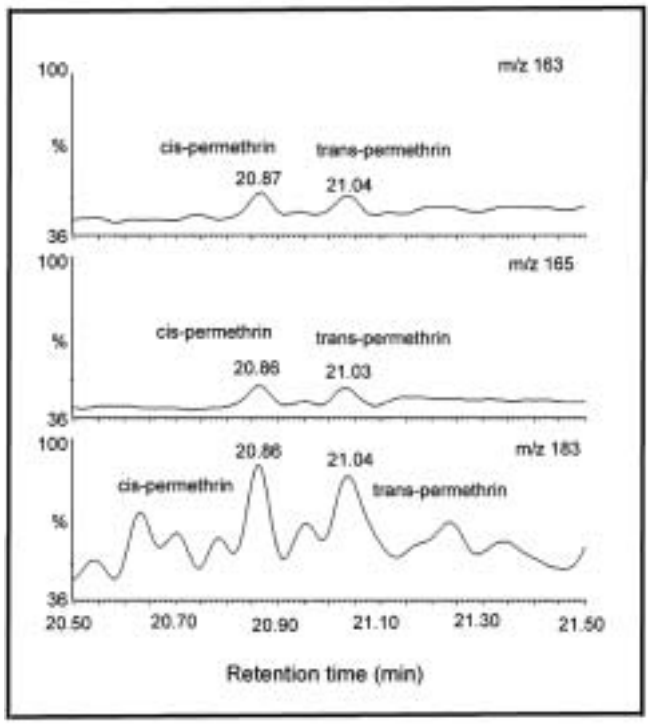

Figure 5. Permethrins in a pest control operator's blood.
Cis–permethrin and *trans*–permethrin were quantified as 0.78 ng/ml (2.3 pg) and 1.1 ng/ml (3.3 pg), respectively.

trans–permethrin. For other six blood samples, both of *cis*– and *trans*– permethrin were lower than the LOQ, while the methabolite of permethrin, 3–PBA were identified and determined from all the seven blood samples. The operator who reported the highest concentration level of permethrin in blood was engaged in pest control operation for the longest time (6 hours) against other operators (0.5 to 2 hours). The biological aspects to those results will be discussed in a separate paper.

4. Conclusion

The present SPE–GC/MS method for the determination of permethrin levels in blood is simple, specific and sensitive. The developed SPE yields 100% recovery of permethrin in a whole blood sample and does not require complicated processing. Both the *cis*– and *trans*–permethrin isomers can be separately quantified even at a sub ppb concentration or with less than 5 pg. However, it will be necessary to shorten the extraction time before the method can be used for routine analysis of a large number of samples.

Acknowledgements

This investigation was part of the "Research and Development Project on the 13 Fields of Occupational Injuries and Illness of the Japan Labour, Health, and Welfare Organization", and was supported by a Grant–in–Aid for Scientific Research (17310033 and 19790404) from the Japan Society for the Promotion of Science.

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