OPTIMIZATION OF THE SWEEP CO-DISTILLATION CLEAN-UP METHOD FOR THE DETERMINATION OF ORGANOCHLORINE PESTICIDE RESIDUES IN PALM OIL

HALIMAH MUHAMAD*; MD PAUZI ABDULLAH; TAN YEW AI* and SOH SHIAU CHIAN****

ABSTRACT

The optimum conditions were developed for the quantitative recovery of organochlorine pesticide residues in palm oil using a commercial sweep co-distillation apparatus. Under the optimum conditions (245°C distillation temperature, 250 ml min^{-1} nitrogen flow rate, 45 min sweep time) and using a trap packed with sodium sulphate and partially deactivated Florisil, the recoveries of 14 organochlorine pesticide residues at ppm and ppb levels in a spiked oil matrix were >80%, with coefficients of variation ranging from 5.6% - 9.9%. However, the recovery for endrin ketone was below 80% with a coefficient of variation of 8.5%. The cleaned-up extracts were quantified by gas chromatography using a micro-electron capture detector with a fused silica capillary column containing a non-polar bonded phase.

Keywords: sweep co-distillation, Florisil, gas chromatography, micro-electron capture detector, organochlorine pesticides.

Date received: 24 August 2003; **Sent for revision:** 26 August 2003; **Received in final form:** 21 June 2004; **Accepted:** 25 June 2004.

INTRODUCTION

Organochlorine (OC) pesticides are now largely banned in Malaysia. However, with their long persistence, their residues may still be in the environment and food chain (Racke, 1993). Over the past decade, public concern over pesticide residues has risen to the point where it has become a significant food safety issue.

Pesticide residue analysis the world over is done using the methods in the U.S. Food and Drug Administration's *Pesticide Analytical Manual* (1994) or AOAC's *Official Methods of Analysis* (1990). These methods are generally time consuming, labour intensive and require large volumes of reagents resulting in them being tedious and expensive (Gretch and Rosen, 1987).

In 1965, Storherr and Watts described sweep co-distillation clean-up of fat samples for pesticide residue analysis. The method can also be used to recover organophosphorus (OP) pesticide residues from spiked crops. A Storherr tube was used to clean up -2 g of crude crop extracts with 89%-101% recovery. Since then, various modifications to the technique have been made.

Neidert and Saschenbrecker (1984), however, had difficulty in detecting low spiked levels. This resulted in the Storherr tube being modified to minimize on-column thermal decomposition of some pesticides. With a silanized Storherr tube packing, a detection limit of <0.005 mg kg^{-1} was achieved.

Luke *et al.* (1984) and Luke and Richard (1984) redesigned the fractionation tube in a revamped commercial version of the sweep co-distillation unit. They also derived the optimum operating conditions for recovering a number of OC and OP pesticides from beef fat with recoveries of 83%-105% and 84%-99%, respectively. Unfortunately, the technique has not been attempted for OC pesticide residues in palm oil.

The objective of this study was to optimize the sweep co-distillation apparatus for determination of OC pesticide residues in an oil matrix. The parameters studied were the distillation

* Malaysian Palm Oil Board,
P. O. Box 10620,
50720 Kuala Lumpur, Malaysia.
E-mail: halimah@mpob.gov.my
** Universiti Kebangsaan Malaysia,
43650 UKM Bangi,
Selangor, Malaysia.

fractionation tube temperature, nitrogen carrier flow, sweep time and eluting solvent mixture.

METHOD

Reagents and Apparatus

- Sweep co-distillation apparatus: Unitrex (Universal Trace Residue Extractor), supplied by Scientific Glass Engineering (SGE) Pte. Ltd., Melbourne, Australia.
- Florisil: 60-100 mesh, 1250°F activated grade (Sigma Chemicals Co.).
- Sodium sulphate: granular, anhydrous AR.
- Solvents: n-hexane and diethyl ether, both AR.
- Trap eluting solvent, 6%: dilute 6 ml diethyl ether to 100 ml with n-hexane.
- Trap eluting solvent, 15%: dilute 15 ml diethyl ether to 100 ml with n-hexane.
- Trap eluting solvent, 50%: dilute 50 ml diethyl ether to 100 ml with n-hexane.
- Nitrogen gas: high purity (for Unitrex and GC).
- Vortex mix: thermolyne, model M37610-26.
- Concentrators: N-Evap model 111.
- Gas chromatography: Hewlett Packard Model 6890 with micro-electron capture detector (μ-ECD) equipped with HP5 as a non-polar stationary phase column containing 95% dimethyl poly siloxane and 5% phenyl methyl siloxane, 30 m x 0.32 mm i.d. and 0.25 μm film thickness. The following operating conditions were used: gas flow (nitrogen) 1.3 ml min^{-1}; average velocity 27 cm s^{-1} using a splitless mode, injector temperature 250°C; detector temperature 325°C and injector volume 1 μl (HP Model 6890 autoinjector). Oven temperature programme: column oven held at 110°C isothermal for 1 min, then rising to 150°C at 25°C min^{-1}, to 260°C at 12°C min^{-1} and to 310°C at 10°C min^{-1} before holding steady for 10 min.
- Pesticide standards: α-BHC; β-BHC; lindane; δ-BHC; α-chlordane; γ-chlordane; p,p'-DDD; p,p'-DDE; p,p'-DDT; dieldrin; endrin; endrin ketone; heptachlor; heptachlor epoxide and methoxychlor (AccuStandard, Inc.).
- Internal standards: tetrachloro-m-xylene and decachlorobiphenyl (Dr Ehrenstorfer-Schafers Laboratory, Ausburg, Germany).

Preparation of Trap Media

Florisil deactivation. The Florisil was washed several times under running tap water and decanted to remove the fine and soluble salts. After drying on a stainless steel tray in an oven or water bath, the Florisil was heated at 650°C for 2 hr. The material was then allowed to cool for 30 min in an oven at 100°C and stored in sealed glass jars. Deactivation was carried out with careful addition of a measured amount of water and leaving the Florisil and water to equilibrate with water overnight. The amount of water added to deactivate the Florisil was determined experimentally, in 0.5% increments from 0.5% to 2.0%. The deactivated Florisil was suitable for use for up to one week and in this study, 1.5% deactivated Florisil was used.

Sodium sulphate. Sodium sulphate was heated in an oven at 130°C for 2 hr and then stored in sealed glass jars in a desiccator.

Trap Preparation

The trap must be freshly packed with deactivated Florisil and granular anhydrous sodium sulphate before use for analysis. The end cone of the glass trap was plugged with silanized glass wool. A small funnel was attached to the other end of the trap and the anhydrous sodium sulphate (1.5 g) carefully poured in. Tapping the trap was vital to ensure uniform packing. Florisil (0.8 ± 0.05 g) was then added on top of the sodium sulphate in the trap. The remainder of the trap was filled with glass wool to make the packing firm. The trap preparation was usually carried out while the unit was being heated to the operating temperature.

Preparation of Standard Stock Solutions

Stock solutions (10 μg ml^{-1}) of α-BHC, β-BHC, γ-BHC (lindane), δ-BHC, α-chlordane, γ-chlordane, p,p'-DDD, p,p'-DDE, p,p'-DDT, dieldrin, endrin, endrin ketone, heptachlor, heptachlor epoxide and methoxychlor were prepared in n-hexane. These solutions were diluted to spike the oil samples to the required concentrations, that is, 0.1-1.0 μg ml^{-1}. All the standard solutions were stored at -20°C in glass bottles with teflon-lined screw caps. Each standard solution contained tetrachloro-m-xylene and decachlorobiphenyl as internal standards with the concentration of each at 0.6 μg ml^{-1}. A stock solution of a mixture of 10 μg ml^{-1} tetrachloro-m-xylene and decachlorobiphenyl was prepared in n-hexane

Plotting the Standard Curves

A stock standard solution (10 μg ml^{-1}) of OC pesticides mixture was diluted to plot a standard curve. The concentrations of the solutions used were 0.008, 0.02, 0.06, 0.2, 0.6 and 1.0 μg ml^{-1}.

Preparation of Spiked Oil

Palm oil (20 g), previously analysed as a blank sample and found to contain no detectable OC pesticide residues, was spiked with 2.0 ml mixed OC pesticide standard in n-hexane to produce concentrations of 1.0, 0.8, 0.4 and 0.1 mg kg^{-1}. Each sample was mixed for 20 min at room temperature to ensure a homogeneous product using a vortex mixture.

Procedure

Operation of the Unitrex apparatus had been described previously by Luke *et al*. (1984). The same procedure was used in this study except for the following changes: fractionation tube temperature increased from 235°C to 245°C; nitrogen flow increased from 230 ml min^{-1} to 250 ml min^{-1}; sweep time increased from 30 min to 45 min and the trap eluting solvent being (50:50) n-hexane:diethyl ether instead of (90:10) n-hexane:diethyl ether. A nitrogen gas stream was used to concentrate the extract to 2.0 ml before its transfer into a vial for automatic injection into the gas chromatography (GC) equipment.

RESULTS AND DISCUSSION

Eluting Solvent Mixture

Before determining the optimum Unitrex operating parameters for recovery of the OC pesticide residues, the eluent suitable for the Florisil traps was established.

The Florisil trap was packed with 0.8 ± 0.05 g activated Florisil (650°C) and 1.5 g sodium sulphate as stated in the *Unitrex Operating Manual* (1993). It was important that the trap was tapped to obtain uniform packing in order to maintain repeatability of the results. Packing of the trap was carried out while the unit was being heated to the operating temperature. A blank palm oil sample was analysed using the Unitrex with the Florisil trap. A 10 μl concentrated OC pesticide standard mixture was injected into the trap and eluted with 12 ml n-hexane-diethyl ether solvent mixture (e, f and g). GC analysis of the concentrated sample extract demonstrated recoveries of >85% for all the OC pesticides except endrin ketone which gave recoveries ranging only from 55% to 65%. The trap eluting solvent of n-hexane:diethyl ether (50:50) was used because of the good recoveries obtained. The extract could also be concentrated more rapidly because diethyl ether has a lower boiling point than n-hexane.

Nitrogen Flow Rate

The first step in optimizing the Unitrex parameters for the OC pesticides recovery was to analyse a series of spiked oil samples at a distillation temperature of 235°C using a range of nitrogen flow rates from 100 to 400 ml min^{-1}. From the recovery data, the flow rate of 250 ml min^{-1} was selected for the optimum recovery of most of the OC pesticides.

Distillation Temperature and Sweep Time

The consequences of increasing the distillation temperature and sweep time were examined. A series of recoveries was carried out on oil samples spiked to higher levels (2-4 mg kg^{-1}). Four different distillation temperatures were examined: 215°C, 225°C, 235°C and 245°C. The distillation tube effluent was collected at selected times (*Figure 1*) by replacing the Unitrex trap with a fresh one. After the trap was eluted, the eluate was concentrated and subjected to GC analysis. The cumulative recovery of each pesticide was plotted against the sweep time at each distillation temperature. The results, as presented in *Figure 1*, illustrate the rapid improvement in endrin ketone recovery with increasing distillation fractionation tube temperature. Recoveries of the remaining OC pesticides were plotted for the single line and single distillation fractionation tube temperature of 245°C for simplicity and ease of comparison. From the recovery (%) obtained (*Figure 1*), a sweep time of 45 min was selected as optimum for the recovery of all the OC pesticides.

Recovery of OC Pesticide Residues from Oil Matrix

The recoveries from the four spiked levels are listed in *Table 1*. The 15 OC pesticides were recovered in the range 55%-112% for all the spiked levels. *Figure 2* shows a typical gas chromatogram of the 15 OC pesticides reference standard solutions used in this study. It was confirmed that no substance in the unspiked oil produced chromatographic peaks that overlapped those of the 15 pesticides (*Figure 3*). The samples were spiked at the typical residual levels and analysed using the optimized Unitrex conditions.

In the recovery results in *Table 1*, all but one of the OC pesticides were >85% recovered with coefficients of variation (CV) of 5.6%-9.9%. The exception was endrin ketone, of which only

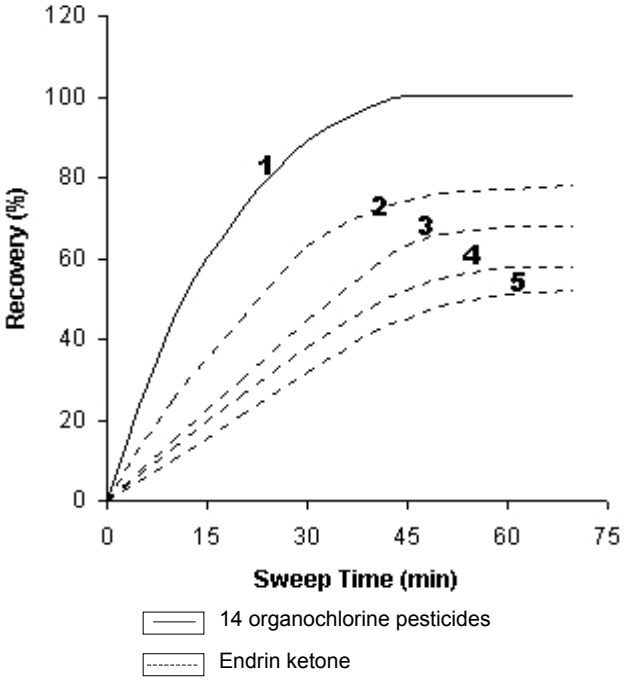

Figure 1. Recoveries of 15 organochlorine pesticides from spiked palm oil samples (using Unitrex apparatus) at different distillation fractionation tube temperatures: 245°C (curves 1 -2), 235°C (curve 3), 225°C (curve 4) and 215°C (curve 5). N_2 flow: 250 ml min^{-1}. Curve identification: 1-14 organochlorine pesticides; 2 - 5 endrin ketone.

55%-65% was recovered with a CV of 8.5%. From *Figure 1*, the most optimum temperature for recovery of the 14 OC pesticides is at 245°C except for endrine ketone. The recovery of endrine ketone in this study was low as expected. There were some reports of low recovery of endrine ketone by other methods. Hopper (1999), using an automated supercritical fluid extraction and an in-line clean-up system developed for OC and OP pesticide residues in fats, reported that 31 pesticides were not recovered through the clean-up procedure and that endrin ketone gave only a low recovery (26%). Syhre *et al.* (1998) also reported low endrin ketone recoveries (20% to 50%) from animal feed and crops using the adsorption material, ENVI-Carb, a graphitized non-porous carbon material. The authors suspected that degradation or the structural properties of the pesticide might have been responsible for the persistently low recovery (Syhre *et al.*, 1998).

Linearity, Limits of Detection and Quantification

Calibration curves were derived from internal standards and the peak areas. The risk of error from the sample preparation was minimal because of the similar chemical behaviour of the internal standards and the unknowns.

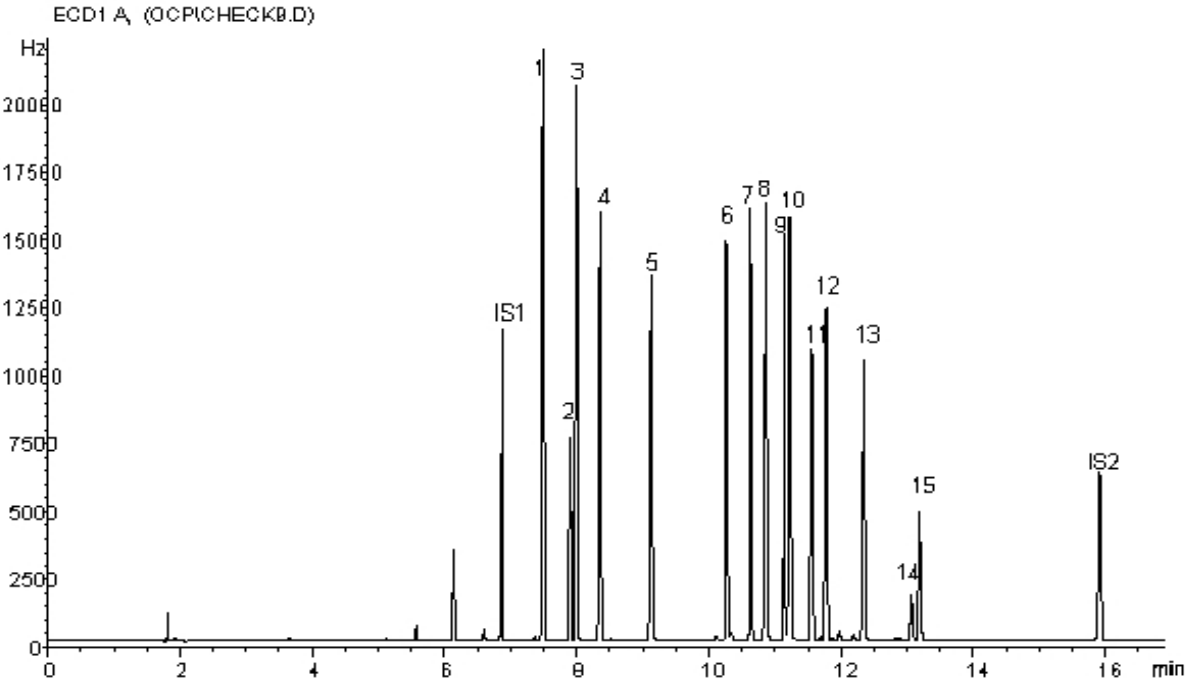

Figure 2. Gas chromatogram of 15 organochlorine pesticide reference standards: 1 µl injection volume of 1 µg ml concentration. Peaks: IS1= tetrachloro-m-xylene; 1= α-BHC; 2= β-BHC; 3= lindane; 4=δ-BHC; 5= heptachlor; 6=heptachlor epoxide; 7= γ-chlordane; 8= α-chlordane; 9= p,p'-DDE; 10= dieldrin; 11= endrin; 12= p,p'-DDD, 13= p,p'-DDT; 14= endrin ketone; 15= methoxychlor; IS2=decachlorobiphenyl.

TABLE 1. MEAN RECOVERIES (%) OF ORGANOCHLORINE PESTICIDES FROM SPIKED PALM OIL SAMPLES (analysed by GC-μECD with the sweep co-distillation clean-up method)

Compound	CAS registry No.	Sample 1[a]: spiked level, 1 mg kg^{-1}	Sample 2[a]: spiked level, 0.8 mg kg^{-1}	Sample 3[a]: spiked level, 0.4 mg kg^{-1}	Sample 4[a]: spiked level, 0.1 mg kg^{-1}	CV, %
α-BHC	319-84-6	93	88	88	86	8.2
β-BHC	319-85-7	106	101	93	106	5.6
Lindane	58-89-9	91	86	86	96	5.6
δ-BHC	319-86-8	105	93	92	86	7.9
Heptachlor	76-44-8	105	91	112	101	7.4
Heptachlor epoxide	1024-57-3	99	99	81	88	8.4
γ-Chlordane	5103-74-2	108	101	85	91	9.5
α-Chlordane	5103-71-9	108	97	85	88	9.4
p,p′-DDE	72-55-9	103	102	95	89	5.9
Dieldrin	60-57-1	109	103	86	90	9.9
Endrin	72-20-8	107	89	89	101	8.2
p,p′-DDD	53-19-0	96	108	97	89	8.6
p,p′-DDT	50-29-3	87	97	85	87	5.9
Endrin ketone	53494-70-5	64	65	60	55	8.5
Methoxychlor	72-43-5	94	94	106	105	6.2

Note: [a] Results from five replicate analyses.

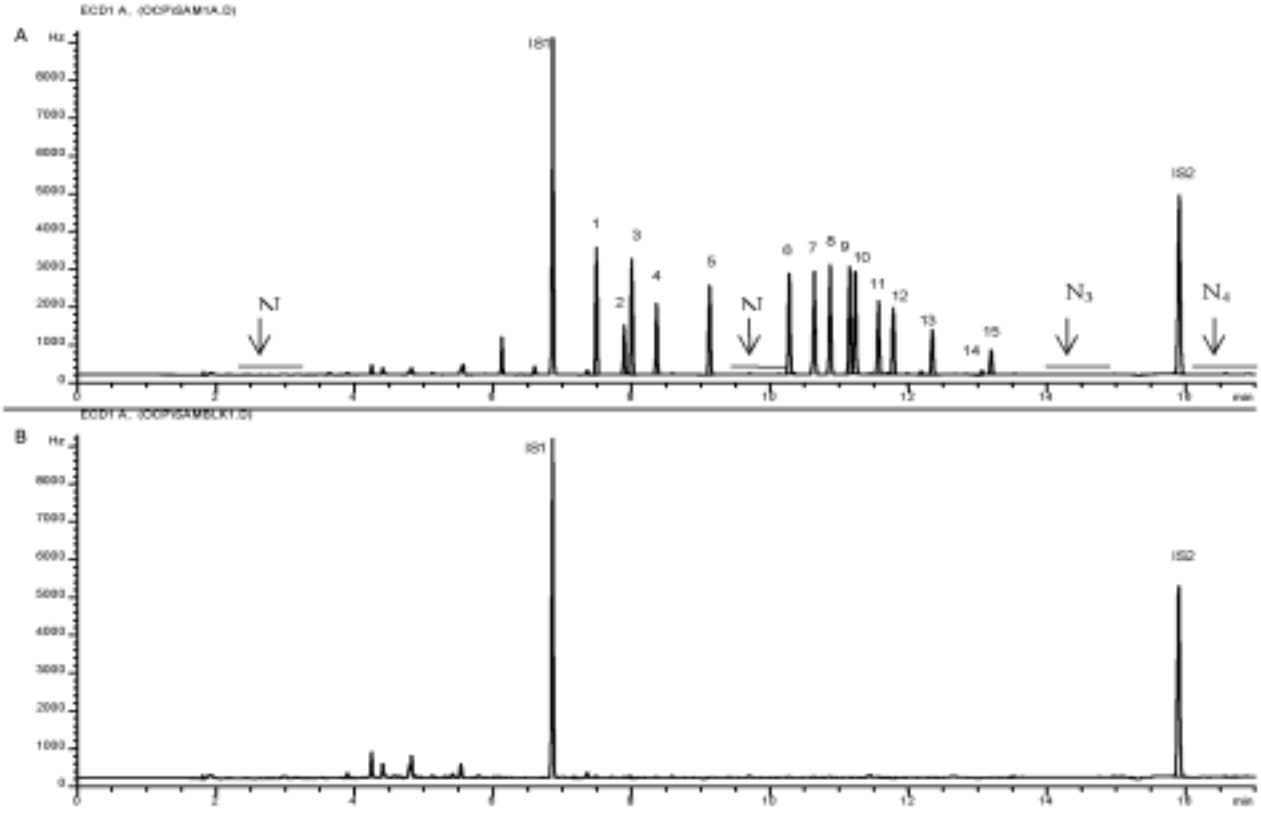

Figure 3. Comparison of gas chromatogram of extracts from Unitrex system with standard reference samples: A= oil fortified with organochlorine pesticide at 0.4 mg kg^{-1}; B= unfortified oil (blank) spiked with internal standards prior to gas chromatography. Peaks: IS1= tetrachloro-m-xylene; 1= α-BHC; 2= β-BHC; 3= lindane; 4= δ-BHC; 5= heptachlor; 6= heptachlor epoxide; 7= γ-chlordane; 8= α-chlordane; 9= p,p′-DDE; 10= dieldrin; 11= endrin; 12= p,p′-DDD; 13= p,p′-DDT; 14= endrin ketone; 15= methoxychlor; IS2= decachlorobiphenyl.

TABLE 2. LIMITS OF DETECTION (LOD), LIMITS OF QUANTITATION (LOQ): CALIBRATION EQUATIONS AND COEFFICIENTS OF DETERMINATION CALCULATED

Compound	LOD, μg ml^{-1}	LOQ, μg ml^{-1}	Equation[b] (r^2)
α-BHC	0.003	0.01	y=1.224x (0.999)
β-BHC	0.01	0.04	y=0.407x (0.999)
γ-BHC (lindane)	0.004	0.01	y=1.178x (0.999)
δ-BHC	0.01	0.05	y=0.689x (0.995)
Heptachlor	0.01	0.04	y=1.034x (0.999)
Heptachlor epoxide	0.009	0.03	y=1.662x (0.999)
γ-Chlordane	0.009	0.03	y=1.652x (0.999)
α-Chlordane	0.009	0.03	y=1.674x (0.999)
p,p′-DDE	0.009	0.03	y=1.529x (0.999)
Dieldrin	0.01	0.03	y=1.046x (0.999)
Endrin	0.01	0.04	y=1.287x (0.999)
p,p′-DDD	0.01	0.04	y=1.357x (0.999)
p,p′-DDT	0.01	0.04	y=1.100x (0.997)
Endrin ketone	0.1	0.3	y=0.170x (0.999)
Methoxychlor	0.03	0.05	y=0.539x (0.999)

Notes: [b]y = Area relationship for pesticide/internal standard; x = concentration of pesticide (μg ml^{-1})/internal standard (μg ml^{-1}).

The calibration points were given weightage on the curves. The CVs were 0.5% to 5.5% and the r^2 values for all the 15 OC calibrations > 0.995. As the CVs for the calibration curves were <20% over the calibration range, linearity through the origin may be assumed (EPA, 1996). *Table 2* shows the linear calibration equations along with their r^2 values and the limits of detection and quantification for each pesticide.

The detection limits for the method were estimated from spiked and recovery experiments with consideration for the normal GC detector sensitivity. The detection and quantification limits for each pesticide were calculated as three and 10 times the chromatographic noise level of the spiked samples, respectively (Bennett *et al.,* 1997; NATA, 1998). The peak-to-peak noise was calculated by averaging the peak-to-peak, high frequency and matrix noise at four areas across the chromatogram (beginning, N_1; middle, N_2 and N_3; and end, N_4), as shown in *Figure 3*. The areas selected did not contain any distinct chromatographic peak.

CONCLUSION

The sweep co-distillation technique offers advantages such as simplicity and savings in labour and solvents. The recovery results indicated the need for having the appropriate operating conditions when the Unitrex system is used for the determination of OC pesticide residues. This method also gave good recoveries of the pesticides studied and was sensitive enough for determining pesticide residues in palm oil.

ACKNOWLEDGEMENT

The authors wish to thank the technical staff of the Pesticides Laboratory, MPOB for laboratory assistance and Universiti Kebangsaan Malaysia for financial support.

REFERENCES

AOAC (1990). *Official Methods of Analysis of AOAC International*. 15th ed., Gaithersburg, MD, secs 970.52-984.21.

BENNETT, D A; CHUNG, A C and LEE, S M (1997). Multiresidue method for analysis of pesticides in liquid whole milk. *J. Assoc. Off. Anal. Chem., 80:* 1065-1077.

EPA (1996). *Office of Research and Development*. U.S. Environmental Protection Agency, Washington, DC, Method 8081A.

GRETCH, F M and ROSEN, J D (1987). Automated sample clean-up for pesticide multiresidue analysis. *J. Assoc. Off. Anal. Chem., 67:* 783-789.

HOPPER, M L (1999). Automated one-step supercritical fluid extraction and clean-up system for the analysis of pesticide residues in fatty matrices. *J. Chromatogr. A., 840:* 93-105.

LUKE, B G and RICHARDS, J C (1984). Recent advances in clean-up of fat by sweep co-distillation. Part 11. *J. Assoc. Off. Anal. Chem., 67:* 902-904.

LUKE, B G; RICHARDS, J C and DAWES, E F (1984). Recent advanced in clean-up of fat by sweep co-distillation. Part 1. *J. Assoc. Off. Anal. Chem., 67:* 295-298.

NATA (1998). *Format and Content of Test Methods and Procedures for Validation and Verification of Chemical Test Methods*. Technical Note 17. December 1998.

NEIDERT, E and SASCHENBRECKER, P W (1984). Improved Storherr tube for assisted and sweep co-distillation clean-up of pesticides, polychlorinated biphenyls and pentachlorophenol from animal fats. *J. Assoc. Off. Anal. Chem., 67:* 773-775.

PESTICIDE ANALYTICAL MANUAL (1994). 3rd ed., Vol. 1, U.S. Food and Drug Administration, Washington, DC. p. 179-180.

RACKE, K D (1993). *Reviews of Environmental Contamination and Toxicology*. Springer-Verlog, New York, Inc. p. 1-150.

SANNINO, A; MAMBRIANI, P; BANDINI, M and BOLZONI, L (1996). Multiresidue method for determination of organophosphorus insecticide residue in fatty processed foods by gel permeation chromatography. *J. Assoc. Off. Anal. Chem., 79:* 1434-1446.

STORHERR, R W and WATTS, R R (1965). A sweep co-distillation method for organophosphate pesticides. *J. Assoc. Off. Anal. Chem., 48:* 1154-1158.

SYHRE, M; HANSCHAMANN, G and HEBER, R (1998). Clean-up procedure for monitoring chlorinated compounds in animal feed and crops. *J. Assoc. Off. Anal. Chem., 81:* 513-518.

UNITREX (1993). *Unitrex™ II Installation and Operating Manual*. Publication No. MN-0004-E. Universal Trace Residue Extractor, Scientific Glass Engineering (SGE) Pty. Ltd., Melbourne, Australia.