

## Determination of Organochlorine Pesticides in Vegetables by Solid-Phase Extraction Cleanup and Gas Chromatography

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### ABSTRAK

Satu kaedah pembersihan yang ringkas dan cepat telah dihasilkan untuk menentukan racun perosak organoklorin pada sayur-sayuran. Sisa baki racun perosak diekstrak dengan aseton dan diklorometana. Ekstrak dibersihkan dengan turus ekstraksi fasa pepejal amina kuaterner dan aminopropil. Racun perosak ditentukan dengan kromatografi gas yang dilengkapi dengan pengesan tangkapan elektron. Perolehan semula untuk enam jenis racun perosak pada tiga jenis sayur-sayuran iaitu lobak merah, timun dan sawi hijau pada aras 0.5 mg/kg adalah di antara 68% dan 109.3%. Keputusan ini adalah setanding dengan yang diperolehi daripada kaedah pembersihan melalui gel silika.

### ABSTRACT

A simple and rapid cleanup procedure has been developed for simultaneous determination of organochlorine pesticides in vegetables. Residues were extracted with acetone and dichloromethane. Extracts were cleaned up by a mixed-mode solid-phase extraction column, quaternary amine and aminopropyl. The pesticides were determined by gas chromatography with electron capture detector. The recoveries of 6 pesticides in three crops, namely carrot, cucumber and green mustard, fortified at 0.5 mg/kg were in the range of 68% to 109.3%. The results obtained were comparable to those obtained by silica gel cleanup method.

**Keywords:** Solid phase extraction, organochlorine, chlorophenyl, conazole pesticides, gas chromatography

### INTRODUCTION

Organochlorine (OC) pesticides were used extensively worldwide in the early 1950s due to their effectiveness, low cost and acute toxicity. The highly lipophilic properties of these pesticides have led to contamination of the environment and the food chain. This has resulted in their restriction or banning in most industrialized countries. But, they remain as important pesticides in agriculture and public health in developing countries, where their use is still permitted. In Malaysia, most of the OC pesticides were no longer registered. The only two OC pesticides currently in use are lindane and endosulfan.

Several multi-residue methods have been developed for the analysis of OC pesticides. Luke *et al.* (1975) developed a rapid multiresidue procedure utilizing florisil cleanup prior to gas chromatography (GC) analysis. The samples were extracted with acetone and partitioned with methylene chloride-petroleum ether. Recoveries for 31 pesticides varied with the polarities of pesticides studied. However, the method was simple and fast with great potential for the analysis of many more compounds. Luke *et al.* (1981) further simplified this method by eliminating the florisil cleanup step by injecting the extract directly into GC with a Hall electrolytic conductivity detector. Good recoveries were obtained for 82 pesticides studied. A rapid multiresidue procedure for fruits, based on methanol extraction was developed by Holland and Mcghie (1983). Water and highly water-soluble co-extractives were removed by partitioning the pesticides into toluene. Further cleaning up was performed by carbon-cellulose-florisil before GC determination. High recoveries were obtained for 30 pesticides studied.

Numerous methods have been developed for the analysis of OC pesticides using solid-phase extraction (SPE) cleanup. Hsu *et al.* (1991) investigated C18 and florisil SPE as replacement for Attagel cleanup procedures for halogenated organic pesticides in broccoli, carrot, celery and orange. The recoveries obtained were comparable to the later method. Lee *et al.* (1991) evaluated the analysis of 7 chlorinated hydrocarbons using reverse phase SPE cleanup for potato, tomato, orange, carrot, broccoli and melon. The method was able to detect at 0.01 ppm level. Holstege *et al.* (1994) reported a multiresidue method, which was able to detect 17 OC pesticides in plant and animal tissue samples. Samples were cleaned up either by automated gel permeation chromatography, silica gel mini column or C18 SPE. Cook *et al.* (1999) investigated the gravity-fed SPE cleanup method for halogenated, phosphorus, sulfur, nitrogen and carbamate pesticides. Results showed that recoveries of 75 - 130 % were possible for GC and LC methods. Odanaka *et al.* (1991) examined SPE C18, C8, C2, CH and pH sorbents for cleanup of melon, apple, cabbage, eggplant and green pepper. The results of these investigations suggested that a significant reduction on the amount of solvents used, less sample matrix interference, an improvement in accuracy and precision were encountered. A method for the determination of 251 pesticide residues in fruit and vegetable samples using GC-MS and LC was described by Fillion *et al.* (2000). Co-extractives are removed by passing the acetonitrile extract through octadecyl (C18), carbon and aminopropyl cartridge SPE cleanup. Limit of detection ranged from 0.02 and 1.0 mg/kg for most compounds studied. Sheridan and Meola (1999) reported a method for detection, quantitation and confirmation of more than 100 pesticides in fruits, vegetables and milk by gas chromatography (GC) with ion trap mass spectrometry (MS/MS) using quaternary amine (SAX) and PSA for cleanup. The sensitivity of this method for many analytes was equal to or lower than those of selective GC detectors such as flame photometric detectors and electrolytic conductivity detectors.

This paper reports the extraction of six pesticides in vegetables using acetone and dichloromethane, cleanup on SPE SAX/NH<sub>2</sub> and determine on GC-ECD. The results were compared to the silica gel cleanup method.

## EXPERIMENT

### *Chemicals and Reagents*

All the chemicals and reagents were of analytical grade. Pesticide standards were obtained from Dr. Ehrenstorfer, Germany. SPE tubes, Isolute SAX/NH<sub>2</sub>, 1 gm, 6 mL, were purchased from International Sorbent Technology.

### *Instrumentation*

A Hewlett-Packard 6890 Gas Chromatograph equipped with Electron Capture Detector was used for the determination of pesticides. GC conditions were set at : Injector temperature, 260°C; detector 280°C; carrier flow (nitrogen) 1.2 mL/min; oven temperature, 100°C (0.2 min), rate 20°C/min to 180°C, rate 2°C/min to 250°C, rate 50°C/min to 300°C (5 min). Pesticides were analysed on an Ultra 1, 25 m × 0.32 mm × 0.5 mm column.

### *Procedures*

The method was validated using four OC pesticides; gamma-BHC, beta-endosulphan, alpha-endosulphan and dieldrin; one chlorophenyl fungicide; chlorothalonil, and one conazole fungicide; hexaconazole. These pesticides were fortified in carrot (*Daucus carota*), cucumber (*Cucumis sativa*), and green mustard (*Brassica chinensis*). Each sample was fortified with 0.5 ppm of each pesticide. Three replicate fortifications for each matrix type were prepared.

Extraction was carried out using a procedure described by Steinwandter (1985). 50 g of sample was homogenised in a blender containing 100 mL acetone, 75 mL dichloromethane and 15 g sodium chloride for three min. The homogenised mixture was allowed to separate into its organic and aqueous layers. The organic layer was transferred to a beaker containing 3 g of sodium sulphate.

For the silica gel method (Steinwandter 1985), which is currently used in our laboratory, 2 mL of extract was transferred to a chromatographic column packed with 10 g of silica gel. The column was eluted with 60 mL of hexane-methylene chloride mixture (4:1 v/v). The eluate was analysed on GC-ECD.

For SPE method, SAX/NH<sub>2</sub> sorbent was used for cleanup. Prior to use, the SAX/NH<sub>2</sub> was conditioned with 10 mL of acetone : petroleum ether (1:2 v/v). 2 mL of extract was transferred to the SPE tube. The SPE tube was eluted with 10 mL of acetone : petroleum ether (1:2 v/v) at flow rate of 1 mL/min. The eluate collected was analysed on GC-ECD.

### *Statistical Analysis*

A t-test was used to determine the significance of difference between the recoveries of the silica gel method and SPE method. They were considered

significantly different when the values of  $t$  (calculated)  $>$   $t$  (critical) at 95% confidence level.

## RESULTS AND DISCUSSION

### *Development of SPE Conditions*

Development of the SPE solvent system was based on several criteria. Solvents, which were hazardous or expensive to dispose of, were not evaluated. A volatile solvent system must be used, as rapid evaporation of a large volume would be required in sample preparation without causing loss of volatile pesticides. The solvent system must be sufficiently polar to extract most polar pesticides. The final extract should have only minimum of matrix co-extractives.

The studies showed that a flow rate of 1 mL/min was sufficient to recover all the pesticides except hexaconazole. It was noted that the SPE should not be left dry after conditioning. This could result in a significant loss of pesticides. The optimum solvent systems for SAX/NH<sub>2</sub> was acetone : petroleum ether (1:2). Increasing the polarity of the solvent would cause lower recoveries for most of the pesticides studied. This is because most of the OC pesticides are non-polar and using solvent with higher polarity will not elute out the pesticides completely.

### *Recovery Studies*

Six pesticides were selected for this study. They were hexaconazole,  $\gamma$ -BHC, chlorothalonil, alpha-endosulphan, dieldrin and beta-endosulphan. Hexaconazole, a conazole fungicide and chlorothalonil, a chlorophenyl fungicide was included in this study because they are detectable by GC-ECD and also commonly used by vegetable farmers. The results of the recovery study are given in Table 1. The recoveries for five pesticides, namely  $\gamma$ -BHC, chlorothalonil, alpha-endosulphan, dieldrin and beta-endosulphan ranged from 70 - 107.7% with coefficient of variation (CV) of 1.5% to 8.3%. Lower recoveries of 44.3% were obtained for hexaconazole.

TABLE 1  
Recovery of organochlorine pesticides

Pesticide	Recovery <sup>(a)</sup> %	CV %
Hexaconazole	44.3	8.3
Gamma-BHC	70.0	6.1
Chlorothalonil	107.7	7.6
Alpha-endosulphan	90.0	3.6
Dieldrin	95.7	1.5
Beta-endosulphan	93.7	2.1

CV = coefficient of variation

(a)  $n = 3$

*Method Validation*

Three types of vegetables, namely carrot, cucumber and green mustard, which represent root, cucurbit and brassica families, were selected for this study. As each group contains different chemical compounds, they may co-elute with the pesticides and hence may cause interferences during the GC analysis.

**Carrot**

Recovery results from spiked carrot samples using SPE SAX/NH<sub>2</sub> and silica gel column chromatography cleanup are shown in Table 2. For the later method, the recoveries ranged from 79.7% to 104% with CV of 3.0% to 5.8%. Comparable results were obtained from SPE SAX/NH<sub>2</sub> cleanup method with recoveries ranging from 68% to 117.7%. When comparing these two methods, the t-value showed that the calculated value was lower than the critical value. Hence, there is no significant difference between these two methods. Chromatograms for the carrot samples using SAX/NH<sub>2</sub> and silica gel cleanup are shown in *Figs. 1* and *2*. Both chromatograms showed no interference peaks, which co-eluted with the pesticides. However, the chromatogram for SPE cleanup contained more unknown peaks especially after the b-endosulphan peak than the silica gel method. This may be due to smaller amounts of sorbent use in SPE cleanup (1 g) than those in silica gel cleanup (10 g). However, the colour of both extracts was yellow. As no interference compound was encountered, no further cleanup was necessary.

TABLE 2  
Recovery of organochlorine pesticides from spiked carrot samples using SAX/NH<sub>2</sub> and silica gel cleanup

Pesticide	SAX/NH <sub>2</sub>		Silica gel	
	Recovery <sup>(a)</sup> %	CV %	Recovery <sup>(a)</sup> %	CV %
Hexaconazole	93.3	2.1	79.7	4.7
Gamma-BHC	109.3	4.6	81.7	5.8
Chlorothalonil	76.3	5.5	95.3	4.0
Alpha-endosulphan	107.3	3.8	102.0	3.6
Dieldrin	68.0	6.2	103.3	4.0
Beta-endosulphan	117.7	3.5	104.0	3.0
AV	95.3		94.3	
SD	19.8		11.0	

AV= average mean

SD = standard deviation

CV = coefficient of variation

(a) n = 3

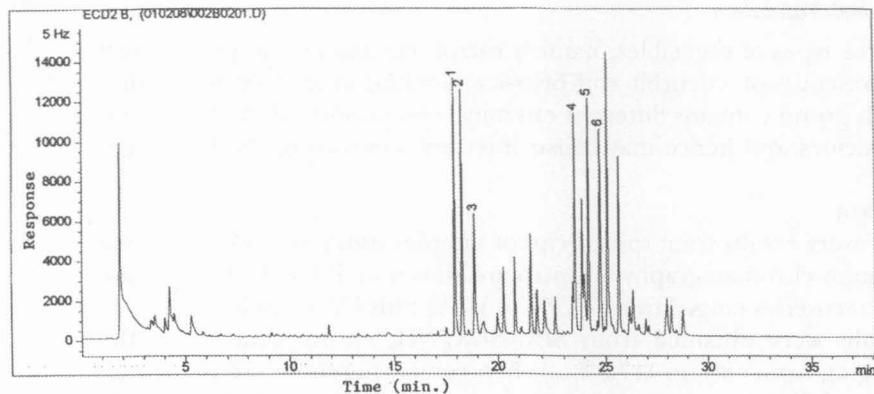


Fig. 1: GC chromatogram of carrot extract after SPE clean-up. Peaks: 1, hexaconazole; 2, gamma-BHC; 3, chlorothalonil; 4, alpha-endosulfan; 5, dieldrin; 6, beta-endosulfan

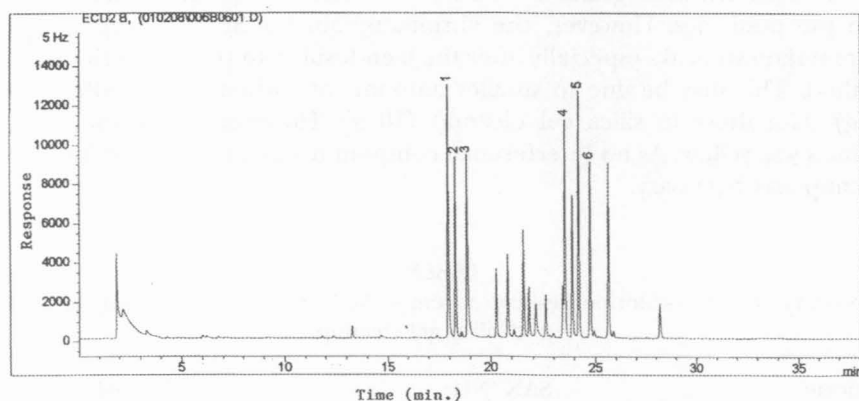


Fig. 2: GC chromatogram of carrot extract after silica gel clean-up. Peaks: 1, hexaconazole; 2, gamma-BHC; 3, chlorothalonil; 4, alpha-endosulfan; 5, dieldrin; 6, beta-endosulfan

### Cucumber

Recovery results of pesticides from fortified cucumber samples using SAX/ $\text{NH}_2$  and silica gel cleanup methods are given in Table 3. Except for hexaconazole, which showed lower recovery of 48%, the recoveries of other pesticides using silica gel method were in the range of 71.0% to 121.3% with CV of 7.0% to 8.5%.

In this study, higher recoveries were obtained for hexaconazole using SPE cleanup method. The recoveries for all the six pesticides evaluated were in the range of 74.0% to 109.7% with CV of 1.0% to 4.0%. Statistical analysis showed that the calculated t-value was lower than the critical t-value. Therefore, there is no significant difference between these two methods.

The chromatograms for silica gel and SPE cleanup methods are shown in Figs. 3 and 4. The extract of cucumber was colourless after the SPE and silica

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TABLE 3  
Recovery of organochlorine pesticides from spiked cucumber samples using SAX/  
NH<sub>2</sub> and silica gel cleanup

Pesticide	SAX/NH <sub>2</sub>		Silica gel	
	Recovery <sup>(a)</sup> %	CV %	Recovery <sup>(a)</sup> %	CV %
Hexaconazole	74.0	1.0	48.0	6.1
Gamma-BHC	95.0	2.0	71.0	7.0
Chlorothalonil	77.7	1.5	109.7	8.5
Alpha-endosulphan	97.3	3.1	115.7	7.6
Dieldrin	105.0	3.0	121.3	7.1
Beta-endosulphan	109.7	4.0	116.7	7.4
AV	93.1		97.1	
SD	14.4		30.2	

AV = average mean

CV = coefficient of variation

SD = standard deviation

(a) n = 3

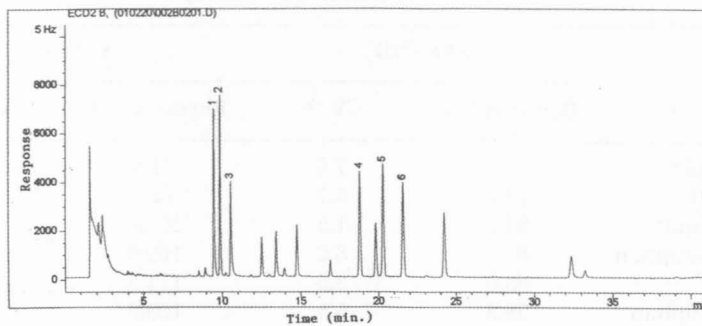


Fig. 3: GC chromatogram of cucumber extract after SPE clean-up. Peaks: 1, hexaconazole; 2, gamma-BHC; 3, chlorothalonil; 4, alpha-endosulfan; 5, dieldrin; 6, beta-endosulfan

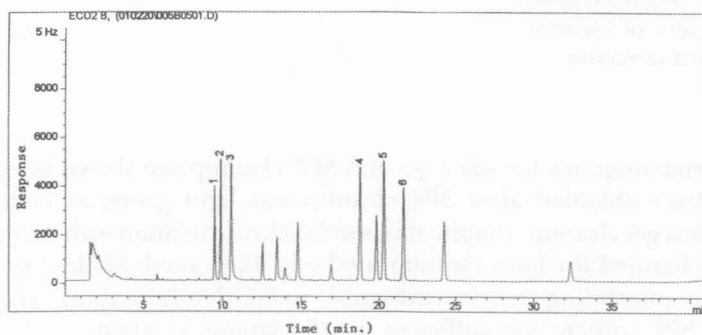


Fig. 4: GC chromatogram of cucumber extract after silica gel clean-up. Peaks: 1, hexaconazole; 2, gamma-BHC; 3, chlorothalonil; 4, alpha-endosulfan; 5, dieldrin; 6, beta-endosulfan

gel cleanups. Similar chromatograms with no interfering peaks were obtained for both cleanup methods. The chromatograms were cleaner as compared to the carrot sample. This was due to fewer compounds present in the cucumber samples which give response to the ECD. It was concluded that a single SPE SAX/NH<sub>2</sub> was sufficient for the cleanup of cucumber sample.

### Green Mustard

The results obtained from spiked green mustard samples using SAX/NH<sub>2</sub> and silica gel cleanup are given in Table 4. The recoveries obtained for silica gel cleanup were in the range of 74.3% to 109.3% with CV of 5.0% to 15.0%. Except for chlorothalonil, which showed lower recoveries of 27.3%, the recoveries for other pesticides obtained by SPE SAX/NH<sub>2</sub> were comparable to the silica gel cleanup method. Their recoveries were in the range of 69% to 98.3% with CV of 5.5% to 8.5%. Statistical analysis showed that the calculated t-value was lower than the critical t-value (excluding chlorothalonil). Therefore, there is no significant difference between these two methods.

TABLE 4  
Recovery of organochlorine pesticides from spiked green mustard samples using SAX/NH<sub>2</sub> and silica gel cleanup

Pesticide	SAX/NH <sub>2</sub>		Silica gel	
	Recovery <sup>(a)</sup> %	CV %	Recovery <sup>(a)</sup> %	CV %
Hexaconazole	69.0	7.6	74.3	5.5
Gamma-BHC	77.7	5.7	72.3	5.0
Chlorothalonil*	27.3	1.5	102.0	7.6
Alpha-endosulphan	84.0	8.2	109.0	7.6
Dieldrin	88.0	8.5	111.3	8.0
Beta-endosulphan	98.3	5.5	109.3	15.0
AV	83.4		95.2	
SD	11.0		20.1	

AV= average mean excluding\*

CV = coefficient of variation

SD = standard deviation

(a) n = 3

The chromatograms for silica gel and SPE cleanup are shown in *Figs. 5* and *6*. The extract obtained after SPE cleanup was light green as compared to yellow in silica gel cleanup. Despite this, similar chromatogram with no interfering peaks was obtained for both cleanup methods. Both methods had successfully removed the interfering co-extractives prior to GC determination. Hence, only one single SPE sorbent was sufficient for the sample cleanup.



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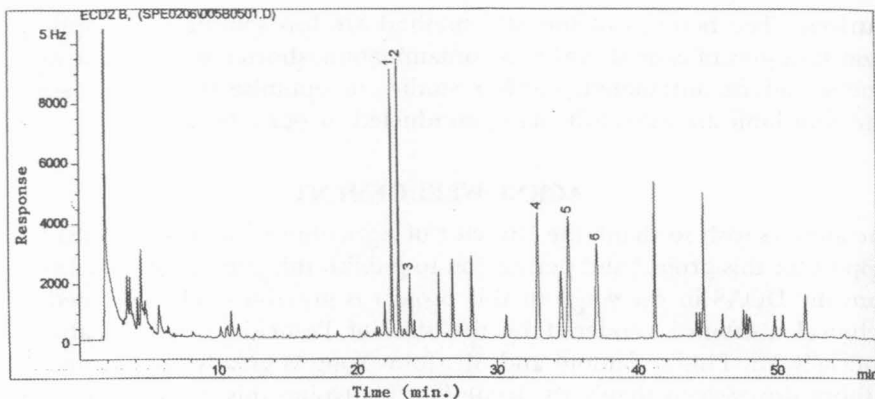


Fig. 5: GC chromatogram of green mustard extract after SPE clean-up. Peaks: 1, hexaconazole; 2, gamma-BHC; 3, chlorothalonil; 4, alpha-endosulfan; 5, dieldrin; 6, beta-endosulfan

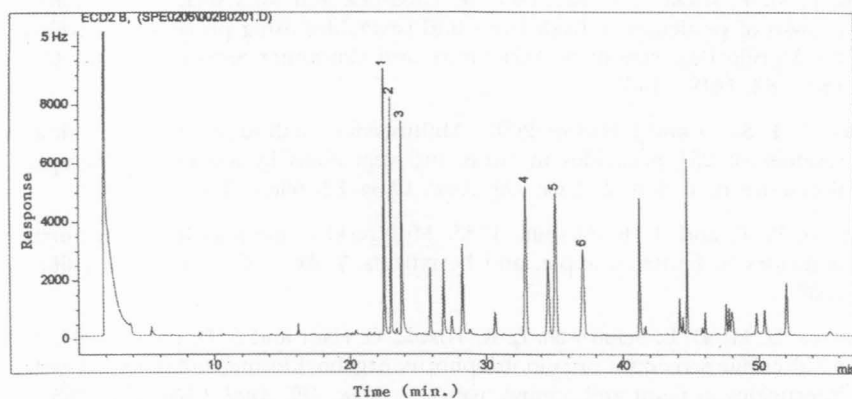


Fig. 6: GC chromatogram of green mustard extract after silica gel clean-up. Peaks: 1, hexaconazole; 2, gamma-BHC; 3, chlorothalonil; 4, alpha-endosulfan; 5, dieldrin; 6, beta-endosulfan

### CONCLUSION

The results from this study showed that SAX/NH<sub>2</sub> is suitable for cleanup of vegetable extracts for OC, chlorophenyl and conazole pesticides. Recoveries obtained were comparable to those obtained from the established silica gel cleanup method. However, lower recoveries occasionally occurred for chlorothalonil and hexaconazole pesticides using both cleanup methods. As these two pesticides are not classified under the OC pesticides, further study needs to be carried out to verify these methods. The results showed that sample cleanup with SPE sorbent were more efficient than silica gel method as less chemicals were used (1 g). It also showed great potential as an alternative method to conventional silica gel for cleanup of vegetables extracts and it is possible to extend the application for the analysis of other pesticides and

matrices. The benefits of the SPE method are less solvent consumption, no hazardous solvent is used, no cross-contamination, shorter analysis time and the process can be automated. Further studies to optimise the sample size and detection limit are currently being conducted in our laboratories.

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#### REFERENCES

- COOK, J., M. P. BECKETT, B. RELIFORD, W. HAMMOCK and M. ENGEL. 1999. Multiresidue analysis of pesticides in fresh fruits and vegetables using procedures developed by the Florida Department of Agriculture and Consumer Services *J. Assoc. Off. Anal. Chem.* **82**: 1419 - 1435.
- FILLION, J., F. SAUVE and J. SELWYN. 2000. Multiresidue methods for the determination of residues of 251 pesticides in fruits and vegetables by gas chromatography with florescence detection. *J. Assoc. Off. Anal. Chem.* **83**: 698 - 713.
- HOLLAND, P. T. and T. K. MCGHIE. 1983. Multiresidue method for determination of pesticides in kiwifruit, apple, and berryfruits. *J. Assoc. Off. Anal. Chem.* **66**: 1003 - 1008.
- HOLSTEGE, D. M., D. L. SCHARBERG, E. R. TOR, L. C. HART and F. D. GALEY. 1994. A rapid multiresidue screen for organophosphorus, organochlorine, and n-methyl carbamate insecticides in plant and animal tissues. *J. Assoc. Off. Anal. Chem.* **77**: 1263 - 1274.
- HSU, R. C., I. BIGGS and N. K. SAINI. 1991. Solid-phase extraction cleanup of halogenated organic pesticides. *J. Agric. and Food Chem.* **39**: 1658 - 1666.
- LEE, S. M., M. L. PAPATHEKIS, H. M. C. FENG, G. F. HUNTER and J. E. CARR. 1991. Multipesticide residue method for fruits and vegetables. *Fresenius Z. Anal. Chem.* **339**: 376 - 383.
- LUKE, M. A., J. E. FROBERG and H. T. MASUMOTO. 1975. Extraction and cleanup of organochlorine, organophosphate, organonitrogen, and hydrocarbon pesticides in produce for determination by gas-liquid chromatography. *J. Assoc. Off. Anal. Chem.* **58**: 1020 - 1026.
- LUKE, M. A., J. E. FROBERG, G. M. DOOSE and H. T. MASUMOTO. 1981. Improved multiresidue gas chromatographic determination of organophosphorus, organonitrogen, and organohalogen pesticides in produce using flame photometric and electrolytic conductctivity detector. *J. Assoc. Off. Anal. Chem.* **64**: 1187 - 1195.
- ODANAKA, Y., O. MATANO and S. GOTO. 1991. The use of solid bonded-phase extraction as alternative to liquid-liquid partitioning for pesticide residue analysis of crops. *Fresenius J. Anal. Chem.* **339**: 368 - 373.

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- SHERIDAN, R. S. and J. R. MEOLA. 1999. Analysis of pesticide residues in fruits, vegetables, and milk by gas chromatography/tandem mass spectrometry. *J. Assoc. Off. Anal. Chem.* **82**: 982 - 990.
- STEINWANDTER, H. 1985. Universal 5-min on-line method for extracting and isolating pesticide residues and industrial chemicals. *Fresenius Z. Anal. Chem.* **322**: 752 - 754.