Toxicol. Res.
Vol. 26, No. 2, pp. 149-155 (2010)

Official Journal of
Korean Society of Toxicology

Available online at http://www.toxmut.or.kr

Determination of Trichlorfon Pesticide Residues in Milk via Gas Chromatography with μ-Electron Capture Detection and GC-MS

Lina Hem[1†], Sathya Khay[1†], Jeong-Heui Choi[1], E.D. Morgan[2], A.M. Abd El-Aty[3] and Jae-Han Shim[1]

[1]*Natural Products Chemistry Laboratory, Division of Applied Bioscience and Biotechnology, College of Agriculture and Life Science, Chonnam National University, Gwangju 500-757, Korea*

[2]*School of Chemistry and Physics, Chemical Ecology Group, Keele University, Staffordshire, ST5 5BG, United Kingdom*

[3]*Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, 12211-Giza, Egypt*

(Received February 23, 2010; Revised May 22, 2010; Accepted May 24, 2010)

The pesticide trichlorfon is readily degraded under experimental conditions to dichlorvos. A method has therefore been developed by which residues of trichlorfon in milk are determined as dichlorvos, using gas chromatography with μ-electron capture detection. The identification of dichlorvos was confirmed by mass spectrometry. Milk was extracted with acetonitrile followed by centrifugation, freezing lipid filtration, and partitioning into dichloromethane. The residue after partitioning of dichloromethane was dissolved in ethyl acetate for gas chromatography. Recovery concentration was determined at 0.5, 1.0, and 2.0 of times the maximum permitted residue limits (MRLs) for trichlorfon in milk. The average recoveries (n = 6) ranged from 92.4 to 103.6%. The repeatability of the measurements was expressed as relative standard deviations (RSDs) ranging from 3.6%, to 6.7%. Limit of detection (LOD) and limit of quantification (LOQ) were 3.7 and 11.1 μg/*l*, respectively. The accuracy and precision (expressed as RSD) were estimated at concentrations from 25 to 250 μg/*l*. The intra- and inter-day accuracy (n = 6) ranged from 89.2% to 91% and 91.3% to 96.3%, respectively. The intra- and inter-day precisions were lower than 8%. The developed method was applied to determine trichlorfon in real samples collected from the seven major cities in the Republic of Korea. No residual trichlorfon was detected in any samples.

***Key words*:** Trichlorfon, Dichlorvos, Milk, Gas chromatography, Mass spectrometry

INTRODUCTION

Milk is an important food in the diet of human beings. The presence of any hazardous chemicals in milk is a threat to human health. Trichlorfon (2,2,2-trichloro-1-hydroxyethylphosphonate, DEP) is an organophosphorus insecticide that has been widely used in protection of field and fruit crops. Since trichlorfon is decomposed to the generally more toxic, organophosphate insecticide dichlorvos, causing serious damage on animals and human beings at pH 6 to 8 in aqueous media (Akhtar, 1982; Doherty *et al*., 1996; Talebpour *et al*., 2006; Grimalt *et al*., 2006; Catalgol *et al*., 2007; Guimarães *et al*., 2007), monitoring the trace level of trichlorfon in food products is essential for human health protection and environmental control (Zhu *et al*., 2008). Animals can accumulate such substances from contaminated feed and water or from insecticide practices in stables. An indirect source of organophosphorus can be represented by animal-derived products (Lopes *et al*., 2006). Trichlorfon is an inhibitor of cholinesterase activity, possible due to *in vivo* conversion to dichlorvos (Grimalt *et al*., 2006). Like all organophosphorus pesticides, trichlorfon is readily absorbed through the skin, subsequently excreted in the urine, and rapidly dechlorinated to dichlorvos in the body.

Some other conventional methods used large volumes of organic solvents and solid phase extracts to extract the sample which was laborious and gave high cost of analysis and laborious. The separation of trace levels of pesticides from animal products in preparation for analysis requires the use of sample preparation techniques that can separate the pesticides from lipids. Even a small amount of lipid can cause deterioration of the column and contamination of the analytical system (Khay *et al*., 2009). Due to the intrinsic ultraviolet-detection (UV) spectral action of trichlorfon, the sensitivity for determination trichlorfon using spectropho-

Correspondence to: Jae-Han Shim, Natural Products Chemistry Laboratory, Division of Applied Bioscience and Biotechnology, College of Agriculture and Life Science, Chonnam National University, 300 Yong-Bong dong, Buk-gu, Gwangju 500-757, Korea
E-mail: jhshim@jnu.ac.kr

†These two authors are equally contributed to this article.

tometry is low (Talebpour *et al*., 2006; Zhu *et al*., 2008). Trichlorfon and dichlorvos are frequently analyzed by gas chromatography (GC). Trichlorfon residues in fruit and vegetables have been determined until now by gas chromatography with flame photometric detection (FPD) and electron capture detection (ECD). Recently, the use of GC coupled to mass spectrometry (MS) has been considered a suitable approach to improve selectivity and sensitivity (Grimalt *et al*., 2006); it is thermally labile and decomposes when analyzed by GC using a heated injector (Ngoh and Cullison, 1996). Analysis of trichlorfon residues has been reported using gas chromatography coupled with FID (Talebpour *et al*., 2006); FPD (Iwata *et al*., 1979; Sheets *et al*., 1982; Maitlen and Halfhill, 1985); NPD (Schultz *et al*., 1971; Ngoh and Cullison, 1996) and mass spectrometry (Aroa *et al.*, 2002; Brito *et al*., 2002; Talebpour *et al*., 2006), and by high performance liquid chromatography (HPLC) (Arao *et al*., 2002; Talebpour *et al*., 2006; Zhu *et al*., 2008).

The purpose of this work was to develop a method for determination of trichlorfon pesticide residue in milk by GC-μECD and confirm it with mass spectrometry (GC-MS). The GC-μECD method was applied to determine residue levels in real sample of fresh milk, from twelve suppliers in seven major cities in the Republic of Korea.

MATERIALS AND METHODS

Chemicals. Trichlorfon standard was kindly provided by the National Agricultural Products Quality Management Services (Gwangju, Republic of Korea). The structural formula of trichlorfon and dichlorvos is shown in Fig. 1. Acetonitrile, acetone, ethyl acetate, *n*-hexane, dichloromethane, and sodium chloride were of HPLC grade and obtained from Merck (Darmstadt, Germany). Sodium sulfate anhydrous was from Yakuri Pure Chemicals Co. LTD (Kyoto, Japan). Water was obtained by filtering deionized water through a 0.45 μm filter with a Waters Millipore (Milford, MA, USA) system.

Standard solutions. A stock solution of 1000 mg/*l* of trichlorfon was prepared in acetone and working solutions were prepared by serial dilution of stock solution with blank sample extracts to 6 different concentrations (25, 50, 100, 150, 200, and 250 μg/*l*). All stock and working solutions were stored at −24°C during study. The residue concentrations were calculated using the calibration curve generated from the peak area versus the working solution concentrations.

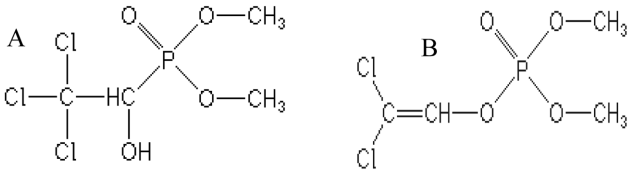

Fig. 1. The structural formula of trichlorfon (A) and dichlorvos (B).

Samples. Fresh milk samples from 12 brands were collected in seven major cities (Incheon, Seoul, Suwon, Busan, Dajeon, Jeonju, and Gwangju) in the Republic of Korea. About one liter of milk homogenized was stored at −24°C until analyzed (within 1 month).

Sample preparation. Ten m*l* of representative fresh milk were introduced into a 50 m*l* centrifuge tube, 5 g of sodium chloride (NaCl) and 30 m*l* of acetonitrile added, the tubes were shaken using a shaking incubator (Dasol Scientific) at 250 rpm for 20 min. The mixtures were centrifuged at 6000 rpm and 4°C for 15 min in order to get clear sample layers. After centrifugation, as much as possible of the clear acetonitrile upper layer was taken and transferred to another tube, 5 g of anhydrous sodium sulfate was added and vortexed for 1 min. The sample was then kept in a refrigerator at −72°C for 20 min. The upper clean layer of acetonitrile (12 m*l*) was mixed with 15 m*l* of saturated sodium chloride, 35 m*l* of water, and partitioned twice with 30 m*l* and then 20 m*l* of dichloromethane, then organic layer was filtered through sodium sulfate. The organic layer was evaporated to dryness in a rotary vacuum evaporator (Bchi Rotavapor R-114; Essen, Switzerland) under 30°C. The residue was dissolved in 2 m*l* of ethyl acetate and filtered through a membrane filterer into a 2 m*l* vial and pesticide was determined by gas chromatography with -ECD.

Instrumentals conditions. GC-μECD: analyses were performed using the Agilent Technologies 7890 A GC system consisted of an autoinjector, model 7683B, and an -ECD. The chromatographic separation was performed using a HP-Ultra 2 (50 m × 0.32 mm, 0.17 m film thickness). The oven temperature was held 100°C for 1 min and increased to 160°C for 1 min by rate of 5°C/min and then increased to 290°C by rate of 30°C/min for 5 min. The temperatures of injection port and detector were maintained at 240°C and 300°C, respectively, the injection volume was 2 μ*l*, and the column was flowed at 2.25 m*l*/min.

GC-MS. Confirmation of trichlorfon was performed with an Agilent technology 6890N with a mass selective detector. The chromatographic separation was performed using a HP-5 MS (30 m × 0.25 mm × 0.25 μm). The oven temperature was held 50°C for 4 min and increased to 300°C by rate of 20°C/min. The temperatures of injection port and detector were maintained at 230°C and 280°C, respectively, and ion source temperature at 230°C.

Recovery tests. The analytical method was validated from blank milk prior to actual analysis. To validate the analytical method, recovery percentage was established by fortification of the pure standard. Recovery experiments were performed by comparing true amount which represent 100% recovery with those of extracted samples, where analysts should have been added at least three different concentrations (Simonelli *et al*., 2007). The samples (10 m*l*) were spiked with trichlorfon in at levels of one half, once, and twice the MRL of trichlorfon in milk (25, 50, and 100 μg/*l*), six replications of each point were done for the recovery tests of trichlorfon in fresh milk. The calibration curve in sample matrix was performed in triplicate at fortification levels of half, one, two, three, four and five times of trichlorfon in the samples.

RESULTS AND DISCUSSION

Due to the intrinsic ultraviolet-detection (UV) spectral action of trichlorfon, the sensitivity for determination trichlorfon using spectrophotometry is low (Talebpour *et al*., 2006; Zhu *et al*., 2008). Gas chromatographic techniques for the detection and determination of trichlorfon residue in animal tissues, plants, and water systems have been reported (Akhtar 1982; Ngoh and Cullison, 1996; Brito *et al*., 2002; Natalia *et al*., 2006). The main purpose of this study was for determining residues of trichlorfon pesticides in milk using gas chromatography with μ-electron capture detection and GC-MS. Because trichlorfon was readily degraded under experimental conditions to dichlorvos, therefore residues of trichlorfon in milk are determined as dichlorvos.

Sample preparation. Acetonitrile extracts usually also contain smaller amounts of co-extractives compared with extracts obtained with other solvents (Anastassiades *et al*., 2003; Dimitra *et al*., 2007; Malone *et al*., 2010). Acetonitrile and sodium chloride were selected for first step extraction because sodium chloride could facilitate separation between acetonitrile and water from milk very well then acetone. After separation, clear acetonitrile upper layer was taken and kept in a refrigerator at −72°C for 20 min to remove lipid of milk. Our sample extraction method is an environment friendly, the organic reagent used is less in amount than tradition extraction with organic solvents, such as liquid-liquid partitioning. The developed method could be carried out much quicker than other previously published method since the method utilizes small volume of samples and extraction solvents, and skips solid-phase extraction to make the method less labor intensive. The method is also very sensitive with all estimated trichlorfon values much lower than its MRL set at 50 μg/*l* by the KFDA (Korea Food and Drug Administration). With less running times, it is a quantitative method and demonstrates good reproducibility and also excellent linearity according to method validations.

Method validation for trichlorfon. Dichlorvos was described instead of trichlorfon in this section because identified peak in the chromatograms is of dichlorvos decomposed from trichlorfon.

Selectivity. The adopted ethyl acetate extraction allowed a chromatographic separation of dichlorvos. The run time was 18 min and the retention time of dichlorvos in the chromatographic condition described was 7.1 min. No interfering peaks were present in the chromatograms (Fig. 2).

Calibration curve linearity. Quantification was accomplished by spiking standards of trichlorfon into blank samples. The linearity achieved for dichlorvos from gas chromatographic was good with range of 25 to 250 mg/*l*, with the correlation coefficient, 0.9988 (Table 1).

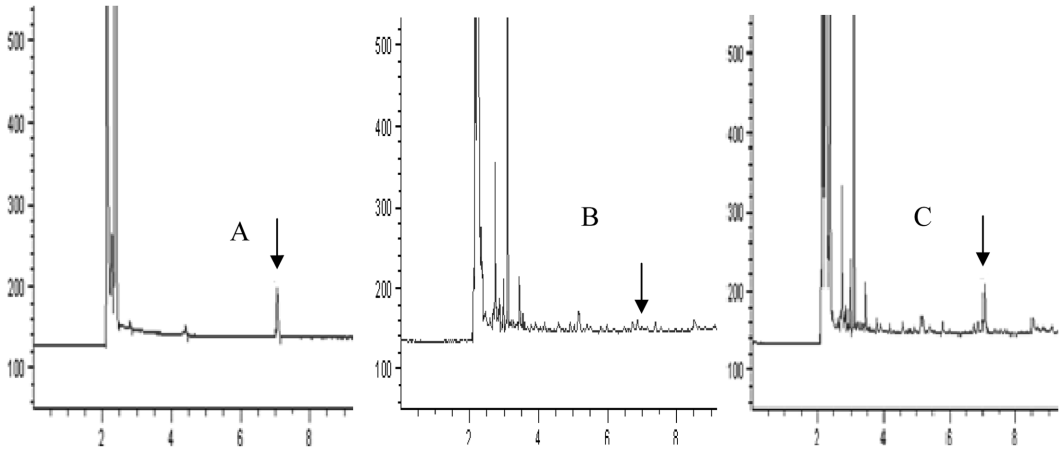

Fig. 2. Chromatograms of dichlorvos decomposed from trichlorfon standard solution (A), blank (B), and fortified milk (C) sample analyzed by GC-μECD.

Table 1. Linearity of dichlorvos detected in milk sample by GC-μECD

Fortified pesticide	Sample	Linear range (μg/*l*)	No. of points	Regression and correlation		
				Slope	Intercept	Correlation coefficient
Trichlorfon	Milk	25~250	6	3.9634	3.02276	0.9988

Table 2. Recovery, limit of detection, limit of quantification of dichlorvos detected in milk sample by GC-μECD (n = 6)

Fortification level (μg/*l*)	Recovery (Mean ± SD, %)	RSD (%)	LOD (μg/*l*)	LOQ (μg/*l*)	MRL (μg/*l*)
25 (MRL)	92.4 ± 4.8	3.6			
50 (1 MRL)	103.6 ± 1.7	6.7	3.7	11.1	50
100 (2 MRL)	96.8 ± 2.7	3.5			

Recovery. Trichlorfon was added to blank milk samples at levels of 25, 50, and 100 μg/*l* and each sample was analyzed, based on the described procedure to revaluate the precision of the analytical method. The results are shown in Table 2. The recovery was measured by comparing peak areas of the spiked samples with those of the related the standard calibration curve. The mean recoveries of 6 replicates were 92.4, 103.6, and 96.8% with relative standard deviations (RSDs) of ± 3.6%, 6.7%, and 3.5%, respectively. Our method meets the requirements of EU guide lines which state that a method can be considered accurate and precise when accuracy data fall between 70 and 110% with RSDs not higher than 20% (SANCO, 2004). The smaller standard deviation obtained indicates good repeatability of the analysis.

Limit of detection (LOD) and limit of quantification (LOQ). According to the ICH Q2B methodology guideline, the limits of detection (LOD) and quantification (LOQ) were calculated as follows:

$$LOD = 3.3\delta/S$$
$$LOQ = 10\delta/S$$

where δ is the standard deviation of blank samples analyzed and S is the slope of the standard curve in matrix. As a result, the LOD and LOQ for dichlorvos were found to be approximately 3.7 μg/*l* and 11.1 μg/*l*, respectively (Table 2).

Intra- and inter-day precision and accuracy. The precision of method was determined as repeatability, and intra-day and inter-day as precision. The precision was expressed in terms of RSD. The inter-day precision was performed on six consecutive days. The intra-day (n = 6) precision and accuracy for trichlorfon spiked levels at 25, 50, and 100 μg/*l* (half, once, and twice time of MRL) were in the range of 89.2% to 91% and 2.6% to 3.3%, respectively. The inter-day (n = 6) precision and accuracy, spiked at the same levels, were ranged from 91.3% to 96.3% and 3.4% to 7.1% (Table 3). Although quantitative decomposition of trichlorfon to dichlorvos is not accomplished and method validation values are calculated as dichlorvos detected, trichlorfon was recovered above at least 90% since dichlorvos decomposed from the fortified trichlorfon was withdrawn by more than 90% in the recovery and intra- and inter-day precision and accuracy.

GC-MS confirmation. GC-MS spectrum of trichlorfon standard injected was the same with those of dichlorvos in the GC library, which presented that total ion current of GC-MS shown the primary and secondary ion were exactly the same to dichlorvos via dehydrochlorination (Fig. 3). The results were similar to Devine (1973), Doherty *et al.* (1996), Na *et al*. (2006), Talebpour *et al*. (2006), and Guimarães *et al*. (2007). Trichlorfon is thermally labile and decomposes when analyzed by GC using a heated injector. Quantitation of trichlorfon has been achieved by monitoring the thermal breakdown products such as dichlorvos, dichloroacetaldehyde, and dimethyl phosphate (Ngoh and Cullison, 1996).

Real sample analysis. The method was later applied to determine the trichlorfon residue levels in real samples from twelve brands products collected from seven major

Table 3. Intra-day and inter-day precision and accuracy of dichlorvos detected in milk sample by GC-μECD (n = 6)

Fortified level (μg/*l*)	Mean calculated concentration (μg/*l*)	Accuracy (%)	Precision (%)
Intra-day precision and accuracy (n = 6)			
25	22.8	91	3.3
50	45	90.1	2.9
100	89.2	89.2	2.6
Inter-day precision and accuracy (n = 6)			
25	22.8	91.3	3.4
50	48.1	96.3	7.1
100	92.9	92.9	4.9

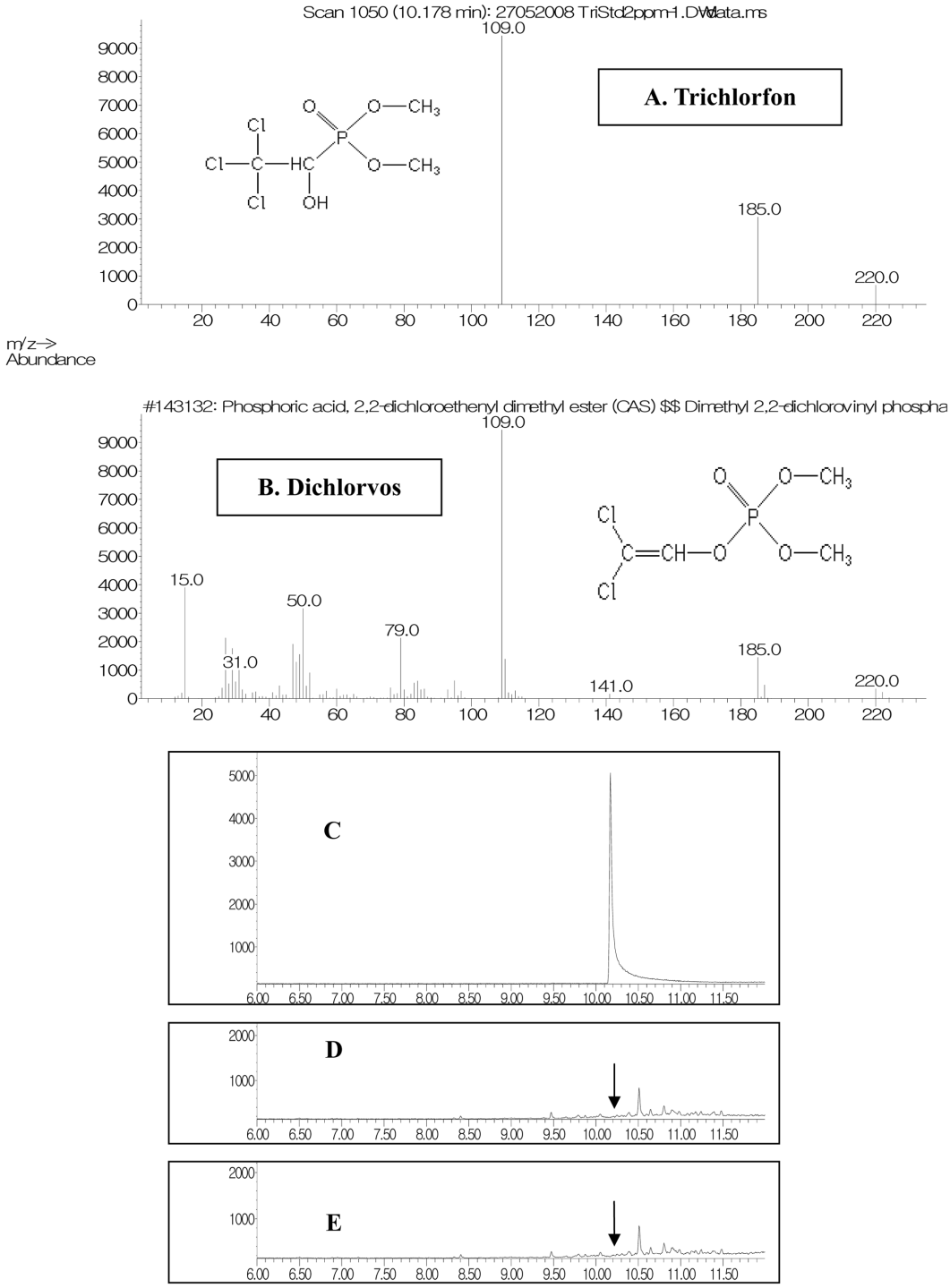

Fig. 3. GC-MS spectrum of trichlorfon standard injected (A) and dichlorvos of library (B), and total ion chromatograms of trichlorfon standard injected (2 ppm) (C), blank milk (D) and real sample (E).

cities (Incheon, Seoul, Suwon, Busan, Dajeon, Jeonju, and Gwangju), in the Republic of Korea. The extraction of each products was performed in triplicate (n = 3). None of trichlorfon was detected in real sample from the results of GC-μECD analyses and GC-MS confirmation (Fig. 3).

CONCLUSION

The method developed for the determination of trichlorfon in milk is simple inexpensive, efficient, and has the important advantage that it requires only small volume of

solvent, especially analysis can be performed without an additional clean up procedure. The method provided a good recovery for trichlorfon with the range of 92.4% to 103.6% with RSD less than 7%. The GC-μECD analysis provided good and efficient analyses for identification of trichlorfon with use of the column HP-Ultra 2. According to GC-MS spectrum, the trichlorfon was changed to dichlorvos while operating in GC. No trichlorfon residues were detected in any of the real samples from twelve supplies in seven major cities in the Republic of Korea.

ACKNOWLEDGMENT

This work was supported by a grant (07052 GITASA 828) of KFDA of the Republic of Korea.

REFERENCES

Akhtar, M.H. (1982). Fate of Trichlorfon in Buffer and Soluble Fraction (105000 g) from Cow and Chicken Liver Homogenates. *J. Agric. Food Chem*., **30**, 551-554.

Anastassiades, M., Lehotay, S.J., Stajnbaher, D. and Schenck, F.J. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "Dispersive solid-phase extraction" for the determination of pesticide residues in produce. *J. AOAC Int*., **86**, 412-431.

Arao, T., Fuke, C., Takaesu, H., Morinaga, Y. and Miyazaki, T. (2002). A case of fatal trichlorfon and methidathion poisoning. *Leg. Med.*, **4**, 182-186.

Brito, N.M., Navickiene, S., Polese, L., Jardim, E.F., Abakerli, R.B. and Ribeiro, M.L. (2002). Determination of pesticide residues in coconut water by liquid-liquid extraction and gas chromatography with electron-capture plus thermionic specific detection and solid-phase extraction and high-performance chromatography with ultraviolet detection. *J. Chromatogr. A*., **957**, 201-209.

Catalgol, B.K., Ozden, S. and Alpertunga, B. (2007). Effects of trichlorfon on malondialdehyde and antioxidant system in human erythrocytes. *Toxicol. In Vitro*, **21**, 1538-1544.

Devine, J.M. (1973). Determination of Trichlorfon [O,O-Dimethyl(2,2,2-Trichloro-1-hydroxyethyl)phosphonate] in Forest Environmental Samples. *J. Agric. Food Chem*., **21**, 1095-1098.

Doherty, A.T., Ellard, S., Parry, M.P. and Parry, J.M. (1996). A study of the aneugenic activity of trichlorfon detected by centromere-specific probes in human lymphoblastoid cell lines. *Mut. Res*., **372**, 221-231.

Grimalt, S., Sancho, J.V., Pozo, O.J., Garca-Baudin, J.M., Fernndes-Cruz, M.L. and Hernndez, F. (2006). Analytical study of trichlorfon residues in kaki fruit and cauliflower samples by liquid chromatography-electrospray tandem mass spectrometry. *J. Agric. Food Chem*., **54**, 1188-1195.

U.S. Department of Health and Human Services, Food and Drug Administration. (1996). Guidance for Industry, Q2B Validation of Analytical Procedures: Methodology.

Guimarães, A.T.B., Silva de Assis, H.C. and Boeger, W. (2007). The effect of trichlorfon on acetycholinesterase activity and histopathology of cultivated fish *Oreochromis niloticus*. *Ecotox. Environ. Safe*., **68**, 57-62.

Hong, X., Qu, J.H., Wang, Y.B., Sun, H., Song, L., Wang, S.L. and Wang, X.R. (2007). Study on the mechanism of trichlorfoninduced inhibition of progesterone synthesis in mouse leydig tumor cells (MLTC-1). *Toxicology*, **234**, 51-58.

Iwata, Y., Dusch, M.E., Carman, G.E. and Gunther, F.A. (1979). Worker environment research: residues from carbaryl, chlorobenzilate, dimethoate, and trichlorfon applied to citrus trees. *J. Agric. Food Chem*., **27**, 1141-1145.

Khay, S., Abd El-Aty, A.M., Choi, J.H., Shin, E.H., Shin, C.H., Chang, B.J., Lee, C.H., Shin, S.C., Jeong, J.Y. and Shim, J.H. (2009). Simultaneous determination of pyrethroids from pesticide residues in porcine muscle and pasteurized using GC. *J. Sep. Sci.*, **32**, 244-251.

Lambropoulou, D.A. and Albanis, T.A. (2007). Methods of sample preparation for determination of pesticide residues in food matrices by chromatography-mass spectrometry-based techniques: a review. *Anal. Bioanal. Chem*., **389**, 1663-1683.

Lopes, R.B., Paraiba, L.C., Ceccarelli, P.S. and Tornisielo, V.L. (2006). Bioconcentration of trichlorfon insecticide in pacu (*Piaractus mesopotamicus*). *Chemosphere*, **64**, 56-62.

Maitlen, J.C. and Halfhill, J.E. (1985). Residues of four pesticides in alfalfa seed and sprouted alfalfa seed following foliar applications. *J. Agric. Food Chem*., **33**, 754-757.

Malone, E.M., Elliott, C.T., Kennedy. D.G. and Regan, L. (2010). Rapid confirmatory method for the determination of sixteen synthetic growth promoters and bisphenol A in bovine milk using dispersive solid-phase extraction and liquid chromatography-tandem mass spectrometry. *J. Chromatogr. B.*, **878**, 1077-1084.

Na, T., Fang, Z., Zhangi, G., Ming, Z. and Cheng, S. (2006). The status of pesticide residues in the drinking water sources in Meiliangwan Bay, Taihu Lake of China. *Environ. Monit. Assess*., **123**, 351-370.

Natalia, F.U., Elisa, B.G. and Alfredo, S.M. (2006). Evaluation of two commercial capillary columns for the enantioselective gas chromatographic separation of organophosphorus pesticides. *Talanta*, **70**, 1057-1063.

Ngoh, M.A. and Cullison, R. (1996). Determination of trichlorfon and dichlorvos residues in shrimp using gas chromatography with nitrogen-phosphorus detection. *J. Agric. Food Chem*., **44**, 2686-2689.

Pagliuca, G., Gazzotti, T., Zironi, E. and Sticca, P. (2005). Residue analysis of organophosphorus pesticides in animal matrices by dual column capillary gas chromatography with nitrogen-phosphorus detection. *J. Chromatogr. A.*, **1071**, 67-70.

European Commission. (2004). Guidelines for the Implementation of Decision 2002/657/EC. SANCO/2004/2726 rev 1, Available online from http://ec.europa.eu/food/food/chemicalsafety/residues/guidelines_2002-657.pdf.

Schultz, D.R., Marxmiller, R.L. and Koos, B.A. (1971). Residue Determination of dichlorvos and related metabolites in animal tissue and fluids. *J. Agric. Food Chem*., **19**, 1238-1243.

Sheets, T.J., Campbell, W.V. and Leidy, R.B. (1982). Fall army worn control and residues of methomyl on coastal Bermuda grass. *J. Agric. Food Chem*., **30**, 532-536.

Talebpour, Z., Ghassempour, A., Zendehzaban, M., Bijanzadeh, H.R. and Mirjalili, M.H. (2006). Monitoring of the insecticide trichlorfon by phosphorus-31 nuclear magnetic resonance (^{31}P

NMR) spectroscopy. *Anal. Chim. Acta*, **576**, 290-296.

Zhu, H.Z., Liu, W., Mao, J.W. and Yang, M.M. (2008). Cloud point extraction and determination of trace trichlorfon by high performance liquid chromatography with ultraviolet-detection based on its catalytic effect on benzidine oxidizing. *Anal. Chim. Acta*, **614**, 58-62.