Chauhan et al. Egyptian Journal of Forensic Sciences (2017) 7:30
DOI 10.1186/s41935-017-0032-8

Egyptian Journal of Forensic Sciences

ORIGINAL ARTICLE Open Access

Method development for determination of residual Chlorpyrifos in the grapes by Tlc-fid

Vimukti Chauhan*, Surbhi Tomar, Yanika Saini and R. M. Tripathi

Abstract

Background: Fruits and vegetables are daily staple food of human community. It is important to include fruits and vegetables in our daily diet to remain healthy and active. Even though fruits and vegetables are healthy, but they are equally prone to pests and diseases which attack them during their time of production as well as storage, thus, degrading their yield and quality. So, to prevent these issues farmers use high amount of pesticides and other products, this enters in our body orally. A large amount of pesticides gets removed from the human body in the form of urine and fecal matter but, still some pesticides (especially chlorpyrifos) are very persistent and can remain in human body for a long term. This study aims at the presentation of a method for the determination of chlorpyrifos from grapes sample by TLC-FID technique. The residue of pesticides was extracted from the sample in ethyl acetate. The grapes sample was macerated, extracted, filtered and analyzed by the proposed method. The analyzed sample showed chlorpyrifos contamination even in the lowest amount taken for analysis.

Results: The TLC-FID technique using mobile phase consist of hexane: acetone (9:1, *v*/v)has been found to be more effective and less tedious as chromarods were used for performing chromatographic separation. Chlorpyrifos were extracted from the samples by liquid-liquid extraction before the analysis. The method developed can be used to detect chlorpyrifos residues in a concentration as minimum as 0.02 mg/Kg.

Conclusion: The regression data was used to calculate the limit of detection and the recovery range was of 97.9% -99.8%. The correlation coefficient calculated from the calibration curve was quite good ($R^2 = 0.9996$).

Keywords: Chlorpyrifos, Grape sample, TLC-fid

Introduction

Chlorpyrifos, (Fig.1) is an organophosphorus insecticide, which is chemically named as thiophosphoric acid O, O′-diethyl ester-O″-(3, 5, 6-trichloro-pyridin-2-yl phosphorothioate) ester (Radišić et al., 2009).

In market it is available under numerous trade names, i.e., Dursban, Lorsban, Pyridane, Pyrinex, Silrifos etc. It is a white or colorless crystal which has a slightly skunky odour, similar to that of rotten eggs or garlic. Chlorpyrifos is insoluble in water but it is soluble in organic solvents like ethyl acetate, acetone, benzene, chloroform, methanol, diethyl ether at room temperature (Radišić et al., 2009).

Chlorpyrifos is the common name for the chemical 0,0-diethyl 0-(3,5,6-trichloro-2-pyridinyl)-phosphorothioate. Chlorpyrifos is used as an insecticide to control many different kinds of pests, including roundworms, termites, mosquitoes etc. Some products which contain chlorpyrifos are especially used in agriculture industry for the treatment of fruits and vegetables (Blakely et al., 2014; Pujeri et al., 2015; Karabasanavar & Singh, 2012). Fruits and vegetables being highly nutritious are produced for local consumption as well as for export purposes. In order to produce higher and better yield, a large amount of insecticide is used by farmers during the entire period of growth (Blakely et al., 2014; Pujeri et al., 2015; Karabasanavar & Singh, 2012). In a study of seven aerobic soils ranging in texture from loamy sand to clay, with soil pH values from 6.0 to 7.4, the soil half life for radiolabeled chlorpyrifos ranged from 11 to 141 days. However, studies have found chlorpyrifos in soils for over one year following application. Since a huge amount of insecticide is used so their irrational and continual use becomes the reason of accumulation of insecticide residues in the primary agriculture products. The continuous use of these insecticides increases the possibility that residues of these compounds could be found in some fruits

* Correspondence: vimuktityagi@gmail.com
Central Forensic Science Laboratory, Chandigarh, India

Springer Open

© The Author(s). 2017 **Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

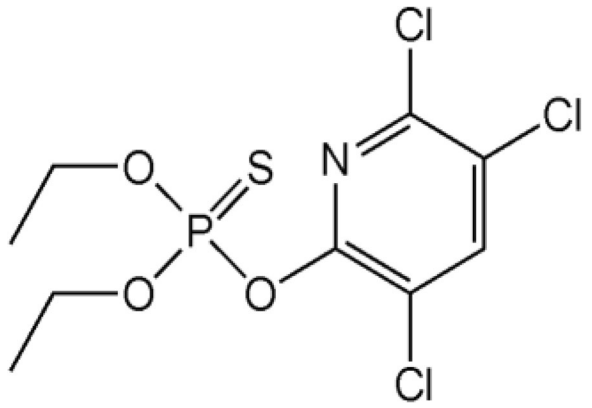

Fig. 1 O, O′- diethyl ester-O″-(3, 5, 6-trichloro-pyridin-2-yl phosphorothioate) ester

and vegetables, thus making this matter a public health and sanitary defense issue (Blakely et al., 2014; Pujeri et al., 2015; Karabasanavar & Singh, 2012). Also, being an organophosphorus insecticide, chlorpyrifos acts as poison if it is touched, inhaled, injected or eaten in any manner (Eaton et al., 2008; Watts, 2013). Chlorpyrifos affects the nervous system. On exposure to chlorpyrifos, it moves to all the parts of the body through metabolic pathways and when the body tries to break it down into its metabolites it gets converted to another form which is called chlorpyrifos oxon. This oxon further binds permanently to the enzymes, which controls the messages that travel between the nerve cells (Eaton et al., 2008; Watts, 2013). Nerves and muscles do not respond correctly when this oxon binds with too many of the enzymes. This causes the body to make more enzymes so that normal nerve function can resume. The body can break down and excrete most of the unbound chlorpyrifos in feces and urine within a few days. Chlorpyrifos that finds its way into the nervous system may stay there much longer. Chlorpyrifos is principally excreted in the urine (Eaton et al., 2008; Watts, 2013).

For human beings to remain healthy, it is important to include fruits and vegetables in their diet for most of the actions taking place in our body. Even though these fruits and vegetables are healthy but they are equally prone to pests and diseases which attack them during their time of production as well as storage thus, degrading their yield and quality (Blakely et al., 2014; Pujeri et al., 2015; Karabasanavar & Singh, 2012). So, to prevent these issues farmers use high amount of pesticides and other products. Even though the use of pesticides has increased the quality and quantity of fruits and vegetables but it has also affected the life of its consumers by causing a large number of health issues to the human beings. Although a large amount of pesticides gets removed from the human body in the form of urine and fecal matter but, still some pesticides (especially chlorpyrifos) are very persistent and can remain in human body for a long term (Eaton et al., 2008; Watts, 2013).

Detailed survey of literature for chlorpyrifos revealed that various techniques have been discovered and used for the assay of chlorpyrifos residue in fruits and vegetables samples. These techniques include TLC (Tewari, 1976), HPLC with UV detection (High Performance Liquid Chromatography) (Richard et al., 2006; Sajjad et al., 2009; Cozma et al., 2011; Devendra et al., 2011; Barkat et al., 2012; Paranthaman et al., 2012; Shailendra et al., 2012; Alamgir et al., 2013; Tordzagla et al., 2013), Liquid chromatography-tandem Mass spectrometery (Steven et al., 2005; Rohan et al., 2012), High Performance Thin Layer Chromatography (HPTLC) (Iqbal et al., 2007; YueY et al., 2008; Akkad & Schwack, 2012; RouhollahD et al., 2012), Gas chromatography–Mass spectrometry(GC-MS) (Paranthaman et al., 2012; Steven et al., 2005; Tomas et al., 2012),Gas Chromatography with Electron Capture detection (GC-ECD) (Devendra et al., 2011; Paranthaman et al., 2012; Mohammad et al., 2010; Subhash et al., 2010), Spectrophotometry (Venugopal et al., 2012), Reflectance near-infrared spectroscopy (Umesh et al., 2012), Chemiluminescence assay (Aifang et al., 2008), immunoassay (Gabaldón & Maquieira, 2007) and Capillary electrochromatography, (Weimin et al., 2010) but in present study an attempt has been made to analyze chlorpyrifos by Thin Layer Chromatography-Flame Ionization Detection technique (TLC-FID) and to validate the method. TLC-FID is a technique which combines the advantages of TLC with the possibility of quantitation using FID. (Cebolla et al., 1998; Bharati et al., 1993; Stephens et al., 1998; Jiang et al., 2008; Ranny, 1987) The separation is made with the TLC Method on chromarods instead of TLC plates and the detection of chlorpyrifos is done with a FID. To separate and identify chlorpyrifos poison, standard solution was prepared and spotted on chromarods with micro dispenser and the rods were made to run in the Hexane: Acetone solvent system and was afterwards subjected to TLC-FID instrument/ IATROSCAN MK-6 s, after which the chromatograms were generated. (Cebolla et al., 1998; Bharati et al., 1993; Stephens et al., 1998; Jiang et al., 2008; Ranny, 1987)Only few methods have been reported for the determination of pesticide residue in leafy vegetables. The methods adopted so far include HPLC with UV detection, (Barkat et al., 2012; Paranthaman et al., 2012; Shailendra et al., 2012) Capillary Chromatography (Weimin et al., 2010) and Gas Chromatography with electron capture detector. (Subhash et al., 2010; Hussain & Samia, 2010)

The current "Joint Meeting on Pesticide Residues" (JMPR) that comprises the WHO Core Assessment Group and the FAO Panel of Experts on Pesticide Residues in Food and the Environment is responsible for reviewing pesticide toxicological data and estimating Acceptable Daily Intakes (ADI), acute reference doses (ARfDs). JMPR

has fixed the ADI (per day per kg body weight) for Chlorpyrifos as 0.01 mg/kg (Bhushan et al., 2013).

Methods

Sampling

Grape samples were collected from the local fruit and vegetable market around Chandigarh, India. The samples were subjected to refrigeration and analysis within a week of collection. All samples were freshly extracted.

Materials

- Acetone, E. Merck
- Hexane, E. Merck
- Ethyl Acetate, E. Merck
- 99% pure Chlorpyrifos Standard, Accustandard Inc.
- Deionized Water
- Sodium Sulfate, Anhydrous
- Grape

Measurement conditions for TLC FID

- Principle of Separation: Thin Layer Chromatography with the use of Chromarods (a special rod coated with a thin layer adsorbent)
- Detection: Hydrogen Flame Ionization Detector (FID) MK-6(s)
- Detection Time: 25 s/scan
- Hydrogen Flow Monitor: Electronic flow meter (digital display)
- Air Flow Monitor: Air flow meter (float type)
- Chromarod Holder: Available for loading 10 Chromarods
- Measuring Modes: Normal scan/Blank scan
- Power: AC 100,120,220&240 V, 50/60 Hz
- Power Requirement: ~50 VA
- Temp. /Humidity: 10~35^0 C/20–80 RH
- Dimensions: ~520x430x265mm MK-6 s~520x430x260
- Weight: ~25Kg MK-6 s: ~23Kg

Extraction of Chlorpyrifos residue from grapes

The efficiency of ethyl acetate extraction method was not tested within this paper as it had been proven to be suitable for a very wide range of pesticide–commodity combinations. The chlorpyrifos pesticide was extracted from grapes samples with optimized extraction method (Hussain & Samia, 2010). 100 g of grapes were macerated with 10–15 g sodium sulfate (anhydrous) and 15 g of sodium bicarbonate in a pestle and mortar to make a fine paste. After maceration, the sample was extracted in 100 mL ethyl acetate at room temperature on mechanical shaker for one hour. The extract (pH 8) was filtered through Teflon filter 0.45 μm and the procedure was repeated by washing the remaining sample 2–3 times with ethyl acetate and concentrated on rotary evaporator. The final volume was made 5 mL with ethyl acetate in a

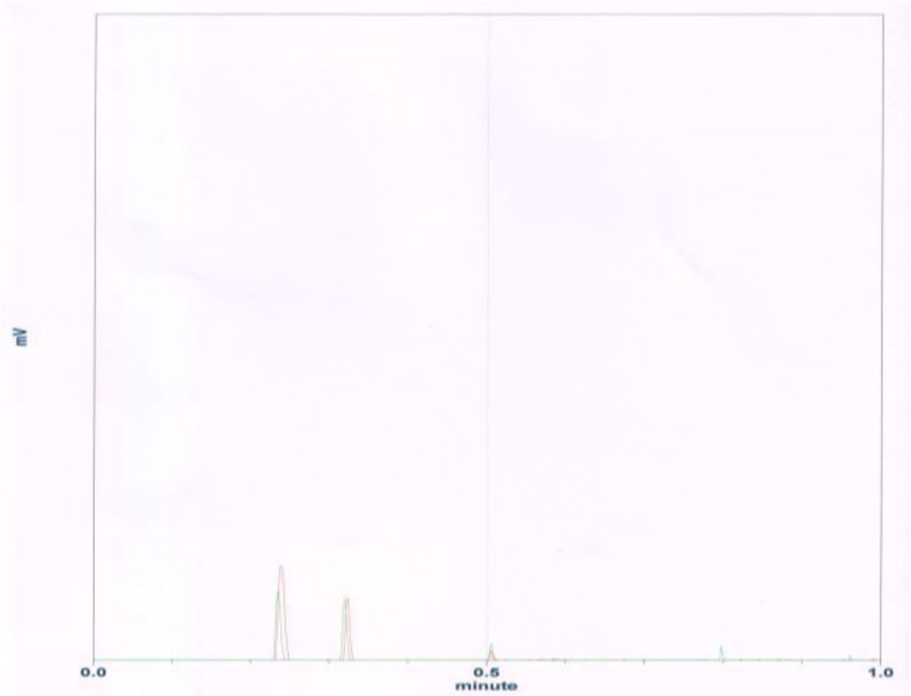

Fig. 2 Chromatogram of chlorpyrifos from grape extract by TLC-FID technique

Table 1 Results of assessment of linearity of TLC-FID method for assay of chlorpyrifos

Concentration (μg/mL)	Concentration as percent of 20 μg/mL	EP peak area as mean of 3 injections	Found Concentration (μg/mL)	% Recovery
5	25	1,988,804	24.912	99.6
10	50	3,965,380	49.479	99.0
15	75	5,894,822	73.459	97.9
20	100	8,013,145	99.788	99.8
Mean ($n = 4$)				99.085
Standard deviation				0.729

glass flask and 20 μL of 5% formic acid in ethyl acetate solution were added to maintain pH 5–5.5,where most acid and base labile pesticide are sufficiently stabilized. The temperature during extraction was maintained between 25 and 33 °C to obtain good extraction efficiency and the temperature was not allowed to exceed 33 ° C.When deep frozen samples were processed the mixture of sample homogenate and the extracting solvent was kept in a water bath at 30°Cto reach the specified temperature range.

Sample preparation

Standard stock solution was prepared by accurately weighing 0.1000 g of standard chlorpyrifos and dissolving it in ethyl acetate and making the volume 100 mL in a volumetric flask.

Working standard solutions of different concentrations were prepared (that is, 25 μg/mL, 50 μg/mL, 75 μg/mL, 100 μg/mL) by diluting the standard stock solution accordingly. Both the stock and working standard solutions were stored in a refrigerator.

TLC-FID instrumentation

Preparation of mobile phase

The mobile phase was prepared by adding 54 ml of hexane in 6 ml of acetone (9:1, *v*/v) (Barkat et al., 2012). The mobile phase was poured in the developing chamber and was lined with filter paper and left as such for 30 min to make the chamber saturated with the vapors of mobile phase.

General requirements

S-III chromarods were used for performing chromatographic separation. Samples were applied using a Micro dispenser (DRUMMOND). Hydrogen and air flow were 160 mL/min and 2–2.5 mL/min respectively.

Procedure

Set of 10 chromarods was previously assembled in a frame. IATROSCAN MK-6 s instrument was started and the rods were subjected to blank scan twice to initially activate the chromarods for further processing. The activated chromarods were kept on chromarod holder. Afterwards, the working standards of different concentrations and grape sample were applied using a micro dispenser. The chromarods were kept in the previously prepared solvent system kept in the development tank and was left as such for development. Chromarods were sequentially passed through H_2 flame in the IATROSCAN FID for peak quantitation at 25 s/scan.

Results and discussions

Since the efficiency of both the techniques gas chromatography/ mass spectrometry (GC/MS) and liquid

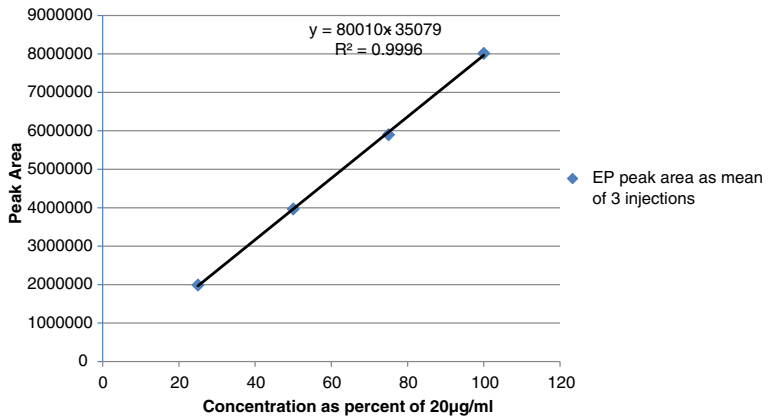

Fig. 3 Calibration Curve

Table 2 System precision results (% RSD) of triplicate sample application of single sample dilution for chlorpyrifos

Concentration (μg/mL)	Concentration as percent of 20 μg/mL	% RSD; ($n = 3$)
5	25	1.00%
10	50	1.00%
15	75	1.00%
20	100	1.00%

chromatography/tandem mass spectrometry (LC/MS/MS) had been tested extensively in the determination of residues of 446 pesticides in fruits and in the method, fortification recovery tests at high, medium, and low levels were conducted on 6 varieties of fruits and vegetables, i.e., apples, oranges, grapes, cabbage, tomatoes, and celery, with average recoveries falling within the range of 55.0–133.8% for 446 pesticides, among which average recoveries between 60.0–120.0% accounted for 99% of the results (Pang et al., 2006). One can observe the effect of surface on the absorption of compounds studied in this work and thus there was no need to repeat those studies.

In the present work, a novel chromatographic methodology was developed to identify Chlorpyrifos, an organophosphorus insecticide residue in grapes samples by TLC-FID technique. The method developed is an alternative analytical method for the detection and confirmation of chlorpyrifos residue. The sample blank was assayed to verify that there are no significant peaks with similar retention times. The blank chromatogram exhibits no peak beyond normal noise level near the retention time of the analyte of interest. To optimize the method various concentrations of analyte were applied using a Micro dispenser and S-III chromarods were used for performing chromatographic separation. Sensitivity of the proposed TLC- FID method was assessed by examining peak interferences from other substances present in grapes extract. This was done by comparing the chromatograms of blank, grapes extract with the authentic chlorpyrifos standard. It was observed that none of the peak appears at the same retention time of pure standard chlorpyrifos. Pesticide residue (chlorpyrifos) was identified from its retention time appeared in grape extract chromatogram (Fig. 2) and confirmed by comparison with authentic standard.

Table 3 Method precision results (% RSD) for 4 individual preparations of a sample for Chlorpyrifos

Concentration (μg/mL)	Peak Area % RSD; (n = 4)
5	1.00%
10	1.00%
15	1.00%
20	1.00%

The linearity was accomplished by making dilutions from stock standard solution. The linearity level, theoretical and actual concentrations as well as r2 value (correlation coefficient) for chlorpyrifos were also calculated. Recovery studies were also performed to examine the efficacy of method. The data used for calculation of the linearity and % recovery is represented as Table 1.

A linear calibration curve (Fig. 3). was also constructed for chlorpyrifos from the linear regression and concentration. The x-axis of the calibration curve represents the concentration whereas the y-axis of the calibration curve represents the area count. The correlation coefficient (r2) from the plotted area response versus concentration for chlorpyrifos is 0.9996 which is ≥0.99. The average percent recovery for chlorpyrifos is 97.9% -99.8% of the amount prepared for 25–100% level.

The accuracy of the method was proven by using spiked solutions that were prepared by spiking in the appropriate amount of analyte of interest into the sample matrix and assayed using a standard. The recovery was within the acceptance criteria and the %RSD among the accuracy dilutions was ≤2.

Afterwards, for each standard concentration, the standard deviation of the "calculated concentrations" was determined and plotted over the actual concentrations of chlorpyrifos. Limit of Detection (LOD) is defined as three times the expected value of the standard deviation of the calculated concentrations at zero concentration. LOD was determined from standard deviation-concentration relationship.

The system precision (reproducibility) and method precision (repeatability) were evaluated using spiked samples. The system precision evaluated the ability of the method to analyze a single sample by applying the sample three times at each level. System precision (reproducibility) results are given in Table 2.

The method precision evaluated the ability of the method to analyze multiple dilutions of sample. This was determined by assaying four individual dilutions. Method Precision (Repeatability) results are given in Table 3.

The %RSD calculated for system precision evaluation ≤2% (Table 2) and that of method precision was also obtained as ≤2% (Table 3). Thus system precision criteria and method precision criteria were met. All acceptance criteria for the linearity of chlorpyrifos were also met.

Table 4 Assay validation sheet

Parameter	Value
Accuracy	99.085 ± 0.729
Slope	80,010
Intercept	35,079

After defining the calibration curve, a calculated concentration was determined for each concentration and analyte based on the slope and intercept of the calibration curve and results are given in Table 4.

The method developed can be used to detect chlorpyrifos residues in a concentration as minimum as 0.02 mg/Kg. The method verification elements of linearity, accuracy, specificity, precision and range met each of the respective elements' acceptance criteria.

Conclusions

The regression data was used to calculate the limit of detection for the method and recovery results were found to be in the range of 97.9% -99.8% The correlation coefficient calculated from the calibration curve and linear relationship was found to be $R^2 = 0.9996$.

The method is precise, accurate and fast, with a standard relative error. The recovery and reproducibility was acceptable for chlorpyrifos based on the matrix spiked standards. The impurities and matrix effects from grapes were minimal and did not interfere with the quantization of any target compound.

The proposed novel method used for the determination of residual chlorpyrifos in the grapes is suitable for analyzing for compliance with current MRLs. In contrast to other TLC based methods, this method can be used alone for the determination of residual chlorpyrifos in sample of unknown origin and can produce reliable and valid results. In order to obtain the best reproducibility and separation, it is recommended to always use freshly activated chromarods and equilibrated solvent/ vapour phase in the developing chamber.

The elaborated method can be tested to cover a wide range of pesticides residue including organophosphorus, carbamate insecticide, synthetic pyrethroid and several other pesticides of various chemical classes from different matrix. Chromarods used in this technique can be subjected to multiple elutions in one or several solvent systems. Further chromarods can also be impregnated with silver nitrate or boric acid to improve separation of quite complex mixtures.

This study evidenced that the proposed novel method may be used in routine analysis of grapes, with low LOD value and good analytical precision.

Acknowledgements

I thank Central Forensic Science Laboratory, Chandigarh and Toxicology Division for permitting me to publish this article.

Ethical approval and consent to participate

"Not applicable" as our manuscript does not report on or involve the use of any animal or human data.

Author's contribution

VC Made substantial contributions to conception and design and interpretation of data; ST Made substantial contributions in acquisition of data or analysis. YS Involved in drafting the manuscript RMT Made substantial contributions in revising it critically for important intellectual content and given final approval of the version to be published. We confirm that this work is original and has not been published elsewhere nor is it currently under consideration for publication elsewhere. In this paper, we report on the detection of chlorpyrifos residue via TLC-FID instrument in the extracted grape sample. This is significant because no pesticide has been detected on this instrument till date and this method will surely be of help in the future of forensic toxicology for the detection of pesticide poisoning in fruit samples. I thank Central Forensic Science Laboratory, Chandigarh and Toxicology Division for permitting me to publish this article. The paper should be of interest to readers in the areas of analytical chemistry and Forensic Toxicology. All authors read and approved the final manuscript.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Availability of data and materials

"The dataset(s) supporting the conclusions of this article is(are) included within the article (and its additional file(s))." in machine-readable format.

Consent for publication

"Not applicable" as our manuscript does not contain any individual persons data.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 28 June 2017 Accepted: 15 December 2017
Published online: 23 December 2017

References

Aifang L, Xiaoyu L, Juan K, Rong H, Chengmou W (2008) Chemilumi -nescence determination of organophosphorus pesticides chlorpyrifos in vegetable. Anal Lett 41(8):1375–1386

Akkad R, Schwack W (2012) Determination of organophosphorus and carbamate insecticides in fresh fruits and vegetables by high-performance thin-layer chromatography-multienzyme inhibition assay. J AOAC Int 95(5):1371–1377

Alamgir M, Chowdhury Z, Shubhra B, Fakhruddin ANM, Islam MN, Alam MK (2013) Determination of cypermethrin, chlorpyrifos and diazinon residues in tomato and reduction of cypermethrin residues in tomato using rice bran. World J. Agri. Res1 (2): 30-35

Barkat AK, Ahmad Z, Sher AK, Zahoor UD (2012) Monitoring pesticide residues in fruits and vegetables grown in Khyber Pakhtoonkhwa. Int. J. Green & herbal Chem 1(3):302–313

Bharati S, Rostum GA, Loberg R (1993) Calibration and standardization of Iatroscan (TLC-FID) using standards derived from crude oils. Org Geochem 22(3–5):835–862

Bhushan C, Bhardwaj A, Misra SS (2013) State of pesticide regulations in India. Centre for Science and Environment, New Delhi

Blakely B, Burtis B, Ludwig G, Isom R, Deiner A, Halsey S (2014) Identifying and managing critical uses of Chlorpyrifos against key pests of alfalfa, almonds, citrus and cotton, CDPR agreement no. 13-C0054, a report submitted to the California Department of Pesticide Regulation, prepared by University of California. Agriculture and Natural Resources:5–192

Cebolla VL, Vela J, Membrado L, Ferrando AC (1998) Suitability of thin-layer chromatography-flame ionization detection with regard to quantitative characterization of different fossil fuel products. I. FID performances and response of pure compounds related to fossil fuel products. J Chromatogr Sci 36:479–486

Cozma A, Bota S, Caraban A, Toderas M, Domuta C, Borza I (2011) Analele Universitatii din Oradea. Fascicula Protectia Mediului, XVII, pp 635–640

Devendra K, Sharma RC, Pratibha C (2011) Estimation of multiclass pesticide residues in tomato (lycopersicon esculentum) and radish (raphanus sativus) vegetables by chromatographic methods. Res J Agri Sci 2(1):40–43

Eaton DL, Daroff RB, Autrup H, Bridges J, Buffler P, Costa LG, Coyle J, Mckhann G, Mobley WC, Nadel L, Neubert D, Schulte-Hermann R, Spencer PS (2008)

Review of the toxicology of Chlorpyrifos with an emphasis on human exposure and neurodevelopment. Critical Review in Toxicology 2:1–122
Gabaldón JA, Maquieira A, Talanta (2007) Development of a simple extraction procedure for chlorpyrifos determination in food samples by immunoassay 71 (3):1001–1010
Hussain Z, Samia S (2010) Determination of pesticides in fruits and vegetables using acetonitrile extraction and GC/MS technique. J Sci Res 40(2):19–29
Iqbal MF, Maqbool U, Asi MR, Aslam S (2007) Determination of pesticide residues in brinjal fruit at supervised trial. J. Ani. Plan Sci 17(1):21–23
Jiang C, Larter SR, Noke KJ, Snowdon LR (2008) TLC-FID (Iatroscan) analysis of heavy oil and tar sand samples. Org Geochem 39:1210–1214
Karabasanavar NS, Singh SP (2012) Occurrence of Chlorpyrifos residues in milk of Tarai region of Uttarakhand, India. Indian Journal of Dairy Science 65(5):370–374
Mohammad AR, Dzolkhifli O, Mohammad ZI, Morshed MM, Rashid KNHB (2010) Supercritical fluid extraction and quantification of chlorpyrifos insecticides in fresh vegetables by gas chromatography with electron capture detection (GC-ECD). Agri Bio J North Am 1(4):548–555
Pang GF, Fan CL, Liu YM, Cao YZ, Zhang JJ, Li XM, Li ZY, Wu YP, Guo TT (2006) Determination of residues of 446 pesticides in fruits and vegetables by three-cartridge solid-phase extraction-gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry. J.AOAC Int 89(3):740–771
Paranthaman R, Sudha A, Kumaravel S (2012) Determination of pesticide residues in banana by using high performance liquid chromatography and gas chromatography-mass spectrometry am. J. Bio. Biotech 8(1):1–6
Pujeri US, Pujar AS, Hiremath SC, Pujari KG, Yadawe MS (2015) Analysis of pesticide residues in vegetables in Vijaypur, Karnataka India. World Journal of Pharmacy and Pharmaceutical Sciences J 4(7):1743–1750
Radišić M, Grujić S, Vasiljević T (2009) Determination of selected pesticides in fruit juices by matrix solid-phase dispersion and liquid chromatography- tandem mass spectrometry. Food Chem 113:712–719
Ranny M (1987) Thin –layer chromatography with flame ionization detector. Holland, Dordrecht
Richard EM, Thomas MP, Theresa AB, John JJ (2006) A simple HPLC method for the determination of chlorpyrifos in black oil sunflower seeds. J Liq Chrom Rel Technol 29(3):339–348
Rohan D, Siddharth T, Padmaja N (2012) Pesticide residue analysis of fruits and vegetables.J. Environ. Chem. Ecotoxicology 4(2):19–28
RouhollahD MS, Hadi E, Gholamabbas M, Davar KR, Davod HS (2012) Detrmination of organophosphorus pesticides (diazinon and chlorpyrifos) in water resources in Barzok. Kashan Zahedan J Res Med Sci 14(10):66–72
Sajjad AB, Niaz AA, Muhammad A, Muhammad RA (2009) Determination of the organophosphorus pesticide in vegetables by high-performance liquid chromatography. Am. Eurasian J. Agric Environ Sci 6(5):513–519
Shailendra SC, Swati N, Nimisha S, Gunjan B, Anjana S (2012) Monitoring of pesticides residues in farmgate vegetables of Uttarakhand, India. Wudpecker J. Agri. Res 1(7):250–256
Stephens FL, Bonner JS, Autenrieth RL, McDonald TJ (1998) TLC/FID analysis of compositional hydrocarbon changes associated with bioremediation, international oil spill conference, p 264
Steven JL, André DK, Maurice H, Peter VB (2005) Validation of a fast and easy method for the determination of residues from 229 pesticides in fruits and vegetables using gas and liquid chromatography and mass spectrometric detection. JAOAC Int 88(2):595–614
Subhash C, Anil NM, Shinde LP (2010) Determination of cypermethrin and chlorpyrifos in vegetables by GC-ECD. Int J ChemTech Res 2(2):908–911
Subhash C, AnilN M, Shinde LP (2010) Determination of cypermethrin and chlorpyrifos in vegetables by GC-ECD. Int. J ChemTech Res 2(2):908–911
Tewari SN (1976) Analytical toxicology. Delhi
Tomas C, Chris S, Veronika B, Lucie D, Kamila K, Jana P, Jana H (2012) Streamlining sample preparation and gas chromatography–tandem mass spectrometry analysis of multiple pesticide residues in tea. Anal Chim Acta 743:51–60
Tordzagla N, AdosrakuK R, Okine NAN (2013) Studies on residues of insecticide used to spray pineapples grown at Samsam in the Amasaman District (Ghana). Int J Environ Sci 3(5):1577–1586
Umesh KA, Phul PS, Kerry BW (2012) Evaluation of a dry extract system involving NIR spectroscopy (DESIR) for rapid assessment of pesticide 209 contamination of fruit surfaces. Am. J. Anal Chem 3(8):524–533
Venugopal NVS, Sumalatha B, Srinivasa Rao B, Veeribabu G (2012) Spectrophotometric determination of organophosphate insecticide (chlorpyrifos) based on diazotisation with anthranilic acid. Malays. J. Anal. Sci 16(2):180–186
Watts M (2013) Chlorpyrifos, Pesticide Action Network Asia &the Pacific. PAN AP 12:1–67
Weimin W, Yimin W, Minmin Z, Liuming Y, Xiaoping W, Xucong L, Zenghong X (2010) Pressurized capillary electrochromatography with indirect 211 amperometric detection for analysis of organophosphorus pesticide residues. Analyst 135:2150–2156
YueY ZR, Fan W, Tang F (2008) High-performance thin-layer chromatographic analysis of selected organophosphorous pesticide residues in tea. J AOAC Int 91(5):1210–1217

Submit your manuscript to a SpringerOpen journal and benefit from:

► Convenient online submission
► Rigorous peer review
► Open access: articles freely available online
► High visibility within the field
► Retaining the copyright to your article

Submit your next manuscript at ► springeropen.com