ISSN 2320-5407 International Journal of Advanced Research (2014), Volume 2, Issue 8, 953-961

ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

COMPARATIVE STUDY ON SOLVENT EXTRACTION METHODS FOR THE DETERMINATION OF PESTICIDE RESIDUES IN VEGETABLES

SUBHASH CHANDRA[1], ANIL N. MAHINDRAKAR[1], M.K. FUGARE[2], L.P. SHINDE[1]

1.P.G. Department of Chemistry, N.E.S. Science College, Nanded-431 605, Maharashtra, India.
2.Department of Physics N.E.S. Science College, Nanded-431 605, Maharashtra, India.

Manuscript Info

Manuscript History:

Received: 15 June 2014
Final Accepted: 17 July 2014
Published Online: August 2014

Key words:
Chlorpyrifos; Cypermethrin; Monocrotophos; Ethyl Acetate, Acetonitrile, GC-MS.

Corresponding Author

M.K. FUGARE

Abstract

A study was carried out to compare the solvent extraction method for the determination of organophosphorus and pyrethroid pesticides in vegetables. Two organophosphorus and one pyrethroid pesticides were extracted by using ethyl acetate and cleaned up with Florisil and activated charcoal column other solvent acetonitrile (MeCN) containing 1% of acetic acid, followed by cleanup of the extract was carried out with primary secondary amine (PSA) and magnesium sulphate and residues were analysed by gas chromatography equipped with mass detector. In this study several reasons, MeCN was found to be the most suitable solvent for extraction of a wide polarity range of pesticide residues from vegetables. MeCN offers advantages in extraction selectivity and compatibility with more diverse analytical techniques compared to EtAc. Pesticides extracted using this method was determined by gas chromatography equipped with mass detector. Better recovery was obtained when the samples extracted with acetonitrile in comparison to ethyl acetate. Recovery of the pesticides were in the range of 80-102.%.

Introduction

Pesticides are a numerous and diverse group of chemical compounds, which are used to eliminate pests in agriculture and households. They enable the quantities and the quality of crops and food to be controlled, and help to limit the many human diseases transmitted by insect or rodent vectors. However, despite their many merits, pesticides are some of the most toxic, environmentally stable and mobile substances in the environment. Their excessive use has a deleterious effect on humans and the environment; their presence in food is particularly dangerous. With their environmental stability, ability to bioaccumulation and toxicity, pesticides may place the human body at greater risk of disease and poisoning [1]. In order to protect consumer's health extensive efforts have been made to the development of new sample preparation techniques that save time, labor and solvent consumption to improve the analytical performance of the procedure.

Numerous methods have been developed for the analysis of OP pesticides in fruits and vegetables. Some of these methods advocate the use of solid-phase extraction (SPE) cartridges. A method using acetonitrile for extraction of pesticide residues in fruits and vegetables was reported [2]. Acetonitrile (MeCN) [3-10] and ethyl acetate (EtAc) [11-15] are two extraction solvents most commonly used in multiresidue methods (MRMs) for the determination of pesticide residues in produce.

The OP insecticides were extracted with methanol dichloromethane (1: 9) and cleaned up using gel permeation chromatograph and silica gel mini columns. Determination of OP pesticides in fruits and vegetables using octadecyl, carbon and aminopropyl cartridges was reported (16). Twenty four pesticides representing different chemical classes (OPPs, OCPs, carbamates and pyrethroids) were determined in fruits and vegetables using GC-MS. The pesticides retained were then eluted with 4 mL of ethyl acetate. Recoveries were found to be between 70% and 110% for most of the pesticides (17). Six common organic solvents for gas chromatographic (GC) analysis of pesticides three of these, acetone, acetonitrile (MeCN) and ethyl acetate (EtAc), represent extraction solvents commonly used in multiresidue methods for determination of pesticides in produce the other three, isooctane, hexane and toluene, often serve as exchange solvents. For several reasons, MeCN was found to be the most suitable solvent for extraction of a wide polarity range of pesticide residues from produce (18).

In this study, it was evaluated two organic solvents commonly featured in either sample preparation (MeCN, acetone, and EtAc) in pesticide multiresidue analysis. The aim of the study to identify he most suitable solvent for the determination of pesticides residues in the vegetable samples.

EXPERIMENTAL

Chemical and reagents

The organic solvent acetic acid, acetonitrile HPLC grade, ethyl acetate, magnesium sulphate and sodium acetate AR grade purchased from E Merck and primary secondary amine purchased from Agilent Technologies. The technical grade pesticide standards were used for standardizations and it were stored in a freezer. Pesticides used for supervised trial chlorpyrifos EC 20% (Chloro-20), cypermethrin 25%EC (Molthrin-25) and monocrotophos SL36% (Monophos-36) purchased from local pesticide suppliers. Anhydrous magnesium sulphate used during residue extraction was maintained at 300^{o}C overnight and kept in air tight container. Polyethylene or PFTE 15ml and 50 ml with screw cap tubes.

Sample collection

Supervised trial was conducted on two vegetables viz. brinjal (Agassim variety) and okra crops (Parbhani kranti variety) at the farmer orchard Vasco-da-Gama, Goa, during summer season (March to June). In supervised trials, chlorpyrifos, cypermethrin and monocrotophos were sprayed with different concentrations (100, 200 and 300g a.i.ha$^{-1)}$ on the brinjal and okra. The sample was not applied any pesticides used as control sample. Samples of okra fruits were collected on 0, 1, 3, 5, 7, 10, 15 days and at harvest after treatment. Residues were estimated by GC-ECD system and reached BDL of 0.010 mg kg-1 on 7th and 15th day in case of single and double dose, respectively The spiked samples were extracted with ethyl acetate and acetonitrile and analysed by GC-MS.

Standard preparation

For preparation of stock solution, standards were dissolved in ethyl acetate and four levels of intermediate standard solution of each pesticide were prepared maintaining the same matrix concentration for the preparation of calibration curve and stored at -4^{o}C in the dark. Working solutions were prepared daily by appropriate dilution with ethyl acetate. The standard solutions were prepared at 0.2 to1.00 mg kg^{-1} concentrations for GC-MS analysis.

Sample extraction and cleanup

Method: 1 Extraction with ethyl acetate

In validation experiments brinjal and okra were used as representative matrices.The fresh samples of brinjal and okra vegetables collected from supervised field were taken for the extraction of pesticide residues. Each vegetable was chopped into small pieces; a representative sample (50gm) was macerated with 5-10gm anhydrous sodium sulphate in blending machine to make fine paste. The macerated sample was extracted with 100 ml of ethyl acetate on mechanical shaker for 1 h; extract was taken for clean up procedure.

Cleanup

In order to achieve the sensitivity required for analysis, the extract of pesticide residues was cleaned up to remove any interfering substances co-extracted with pesticide residues. For this purpose, a Florisil column and charcoal column was used.The clean-up of pesticides were carried out by using column chromatography. Column (60cm x 22mm) was packed with, Florisil and activated charcoal (5:1 w/w) in between the two layers of anhydrous sodium sulphate. The column was then filled with Florisil that had been activated at 150^{o}C for at least 24 hours followed by charcoal and anhydrous sodium sulphate. Just prior to use, the column containing adsorbents was washed with ethyl acetate before loading the sample and flow rate was adjusted to get optimum recovery of the analyte. Extract was eluted with 125 ml mixture of ethyl acetate and hexane (3:7 v/v). After concentrating the eluate on rotary evaporator, final volume was made to 2ml for analysis by gas liquid chromatography (GC).

Method: 2 Extraction with acetonitrile

The fresh brinjal and okra 2 kg each samples were taken for the extraction of pesticide residues. The samples were macerated to make paste with Philips mixer (equipped with stainless steel knives), a 15 g portion of the homogenized sample was weighed into a 50 ml polytetrafluoroethylene (PTFE) tube added 15 ml of acetonitrile containing 1% acetic acid (v/v). Then, 6 g MgSO4 and 2.5 g sodium acetate trihydrate (equivalent to 1.5 g of anhydrous form) were added, and the sample was shaken forcefully for 4 min and kept in ice bath. The samples were then centrifuged at 4000 rpm for 5 min and 6 ml of the supernatant were transferred to a 15 ml PTFE tube to which 900 mg MgSO4 and 300 mg PSA were added. The extract was shaken using a vortex mixer for 20 s and centrifuged at 4000 rpm again for 5 min, approximately 2ml of the supernatant were taken in a vials. This extracts were evaporated to dryness under a stream of nitrogen and reconstituted in n-hexane in auto sampler tube for the GC-MS analysis.

Instrumentation

The analysis of pesticide was carried out by an integrated system of gas chromatography, equipped with automatic injection system and coupled to a mass spectrometric system with ion trap analyser. Varian CP-3800 GC, Saturn-2200 mass spectrometer with auto injector CP-8410 was used for analysis. The mass spectrometer was auto tuned using perfluorotributylamine (PFTBA). The separation of pesticide was done in a 30 meter length, 0.25 mm internal diameter and 0.25 µm film thickness coated with 5% phenyl-95% methylpolysiloxane Varian VF-5MS column. Helium was used as the carrier gas at 9.6 psi pressure and 1 ml min-1 flow. The injector was used at constant temperature 280^0C. The initial oven temperature was 80^0C (4min. isothermal) to 180^0C (at 20^0C min^{-1}) to 250^0C (at 2^0C min^{-1}) to 280^0C (at 10^0C min^{-1}) isothermal for 4 minutes. The injection volume was 1µL in splitless mode. The temperature of ion trap, manifold and transference line was 220^0C, 50^0C and 300^0C respectively. The mass spectrometer was used in SIM mode under electron impact at 70 eV and scan time 1 second. The computer that controlled the system also held a GC-MS library specially created for the target analytes under our experimental conditions. The mass spectrometer was calibrated weekly with perfluoro-tributylamine. Helium (99.999%) at a flow-rate of 1 ml min^{-1} was used as carrier and collision gas.

Method Validatation

In order to check the feasibility of the GC-MS method for the analysis of pesticide residues in fresh sample extracts, it was validated using control brinjal and okra extracts.

Limit Of detection (LOD) and limit of quantitation(LOQ)

The limit of detection (LOD) is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. It may be expressed as a concentration that gives a signal to noise ratio of 3 with reference to the background noise obtained from blank sample. The lowest concentration of the pesticide that gave peak area five times greater than background level was considered as LOD, and the values are in the range of 0.001-0.004 $mgkg^{-1}$. Limit of Quantitation (LOQ) is the lowest amount analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. A signal-to-noise ratio of 10 can be taken as LOQ of the method. The LOQ values are in the range of 0.005-0.020 mg kg^{-1} (table:1).

Linearity

The linearity of the calibration plots was studied using calibration solutions prepared in the control/blank sample extract. The correlation coefficients of analytical curves were near 0.99, with linearity for each compound, which allows the quantitation of these compounds by the method (table:2)

Recovery

The accuracy of an analytical method is the closeness of experimental results obtained by that method to true value. The accuracy of the method was estimated through recovery experiment. For this purpose, control samples (brinjal and okra) were spiked with a 1.0mg kg^{-1} of pesticides. The concentration of each pesticide in the final extracts was calculated (table 3). The average recoveries of pesticide residues in brinjal and okra samples were 80.0 to 102.0 %.

Application of extraction method to the supervised trial samples

In order to test the feasibility of the solvent extraction methods for the analysis of pesticide residues in the supervised trial samples of vegetables (brinjal and okra) using gas chromatography and mass spectrometer. The comparative solvent extraction and recovery of pesticides residue from supervised trails is given in the (table: 4 to 9).

RESULTS AND DISCUSSION

Acetonitrile and ethyl acetate are two extraction solvents most commonly used for isolation of multiple pesticide residues from produce and each of them has been demonstrated to give acceptably high recoveries of a wide range of pesticides (5-8, 9-12,14). In contrast to MeCN, EtAc is practically immiscible with water (19) which can easily be removed from EtAc extracts by a drying agent (usually anhydrous Na2SO4). In the case of MeCN, the use of a proper combination of salts (such as anhydrous MgSO4 and NaCl in the QuEChERS method (3) can provide a well-defined phase separation without dilution and high recoveries including rather polar pesticides. Moreover, anhydrous MgSO4 removes residual water remaining in organic phase after the partitioning step more efficiently from MeCN. In terms of extraction selectivity, MeCN isolates much less lipophilic compounds from samples in comparison with acetone and EtAc (amount of lipophilic co-extractives decreases in the order: EtAc > acetone > MeCN (20). For example, dispersive-SPE clean-up used in the QuEChERS method (5) for removing of co-extractives from MeCN extracts makes the clean-up step relatively fast, inexpensive, and convenient. An additional important advantage of MeCN versus EtAc is its compatibility with reversed-phase liquid chromatography (LC), which offers a possibility to analyze both GC- and LC-amenable analytes in the same extract. To summarize, the three extraction solvents can be ordered according to their suitability for sample preparation in analysis of pesticide residues in produce as follows: MeCN >EtAc . In terms of cost, MeCN is approx. 1.4 and 1.7 times more expensive than a similar grade EtAc, respectively. MeCN is also more toxic, but its negative impact on human health and the environment is much lower than in the case of chlorinated solvents (such as dichloromethane), which are still used in routine practice. According to the US Environmental Protection Agency (EPA), MeCN is not classified as a human carcinogen and is not persistent in the environment. Moreover, the lower volatility of MeCN reduces analyst exposure, which can be further minimized by following proper handling procedures (3).

.

Table 1: Molecular formula, retention time, LODs and LOQs of monocrotophos, chlorpyrifos and cypermethrin.

Compound	Molecular formula	RT (min)	LoDs (mg kg^{-1})	LoQs (mg kg^{-1})
Chlorpyrifos	$C_9H_{11}Cl_3NO_3PS$	25.12	0.003	0.009
Cypermethrin	$C_{22}H_{19}Cl_2NO_3$	31.32	0.003	0.009
Monocrotophos	$C_7H_{14}N O_5P$	17.89	0.004	0.012

Table 2: Quantitation ion, confirmation ion and calibration range of chlorpyrifos cypermethrin and monocrotophos

Compound	Quantitation ion	Confirmation ion	Calibration range (mg kg^{-1})	Correlation coefficient	Coefficient of variation (n = 5) %
Chlorpyriphos	97	314	0.02-1.00	0.991	6.8
Cypermethrin	181	127	0.02-1.00	0.992	6.2
Monocrotophos	127	98	0.02-1.00	0.988	6.4

Table 3: Recovery of pesticides in the spiked samples.

Sample	Pesticide	Concentration (mg kg^{-1})	Recovery %	Coefficient of variation (n = 5) %

Brinjal	Chlorpyrifos	1.0	92.50	6.8
Brinjal	Cypermethrin	1.0	90.22	6.2
Brinjal	Monocrotophos	1.0	102.00	6.4
Okra	Chlorpyrifos	1.0	90.50	6.8
Okra	Cypermethrin	1.0	80.20	6.2
Okra	Monocrotophos	1.0	101.80	6.4

Table: 4 Extraction of chlorpyrifos residue using different solvent system in brinjal samples collected from supervised field sprayed with 100, 200 and 300g a.i. ha^{-1}

Days after treatment	Recovery of residues (mg kg^{-1})					
	Sample extracted with ethyl acetate residues%			Sample extracted with acetonitrile residues%		
	dose (100 g a.i. ha^{-1})	dose (200 g a.i. ha^{-1})	dose (300 g a.i. ha^{-1})	dose (100 g a.i. ha^{-1})	dose (200 g a.i. ha^{-1})	dose (300 g a.i. ha^{-1})
0 (1hr)	0.358	0.678	0.874	0.362	0.679	0.876
1	0.223	0.404	0.501	0.229	0.409	0.501
3	0.127	0.240	0.284	0.129	0.242	0.288
5	0.049	0.101	0.138	0.051	0.104	0.141
7	0.033	0.045	0.058	0.035	0.047	0.059
9	0.005	0.017	0.024	0.005	0.018	0.026
11	0.002	0.008	0.010	0.002	0.010	0.012
13	BDL	0.002	0.006	BDL	0.002	0.007
15	-	BDL	0.004	-	BDL	0.004
17			BDL			BDL

Table: 5 Extraction of cypermethrin residue using different solvent system in brinjal samples collected from supervised field sprayed with 100, 200 and 300g a.i./ha.

Days after treatment	Recovery of residues (mg kg^{-1})					
	Sample extracted with ethyl acetate residues%			Sample extracted with acetonitrile residues%		
	dose (100 g a.i. ha^{-1})	dose (200 g a.i. ha^{-1})	dose (300 g a.i. ha^{-1})	dose (100 g a.i. ha^{-1})	dose (200 g a.i. ha^{-1})	dose (300 g a.i. ha^{-1})
0 (1hr)	0.339	0.657	0.858	0.340	0.661	0.858
1	0.203	0.382	0.483	0.207	0.382	0.488
3	0.106	0.221	0.265	0.106	0.224	0.266
5	0.028	0.085	0.119	0.029	0.088	0.119
7	0.014	0.028	0.038	0.015	0.030	0.038
9	0.005	0.011	0.021	0.005	0.013	0.023
11	BDL	0.004	0.010	BDL	0.004	0.011
13	-	BDL	0.004	-	BDL	0.004

15	-	-	BDL	-	-	BDL
17	-	-	-	-	-	-

Table: 6 Extraction of monocrotophos residue using different solvent system in brinjal samples collected from supervised field sprayed with 100, 200 and 300g a.i./ha

Days after treatment	Recovery of residues (mg kg^{-1})					
	Sample extracted with ethyl acetate residues%			Sample extracted with acetonitrile residues%		
	dose (100 g a.i. ha^{-1})	dose (200 g a.i. ha^{-1})	dose (300 a.i. ha^{-1})	dose (100 a.i. ha^{-1})	dose (200 a.i. ha^{-1})	dose (300 a.i. ha^{-1})
0 (1hr)	0.379	0.689	0.889	0.389	0.689	0.891
1	0.244	0.424	0.518	0.248	0.429	0.521
3	0.148	0.262	0.295	0.148	0.264	0.297
5	0.071	0.122	0.145	0.073	0.128	0.149
7	0.038	0.058	0.068	0.038	0.058	0.068
9	0.021	0.034	0.032	0.024	0.039	0.032
11	0.008	0.008	0.017	0.008	0.009	0.018
13	BDL	0.002	0.008	BDL	0.004	0.008
15	-	BDL	0.004	-	BDL	0.004
17			BDL			BDL

Table: 7 Extraction of chlorpyrifos residue using different solvent system in okra samples collected from supervised field sprayed with 100, 200 and 300g a.i./ha.

Days after treatment	Recovery of residues (mg kg^{-1})					
	Sample extracted with ethyl acetate residues%			Sample extracted with acetonitrile residues%		
	dose (100 g a.i. ha^{-1})	dose (200 g a.i. ha^{-1})	dose (300 a.i. ha^{-1})	dose (100 a.i. ha^{-1})	dose (200 a.i. ha^{-1})	dose (300 a.i. ha^{-1})
0 (1hr)	0.385	0.695	0.874	0.389	0.696	0.874
1	0.254	0.432	0.520	0.258	0.432	0.520
3	0.152	0.272	0.292	0.155	0.275	0.296
5	0.073	0.129	0.156	0.078	0.129	0.156
7	0.061	0.072	0.072	0.067	0.075	0.074
9	0.029	0.034	0.044	0.029	0.036	0.044
11	0.013	0.023	0.034	0.015	0.025	0.035
13	0.008	0.014	0.026	0.008	0.016	0.028
15	BDL	0.007	0.012	BDL	0.009	0.013
17		BDL	0.009		BDL	0.009
19			BDL			BDL

Table: 8 Extraction of cypermethrin residue using different solvent system in okra samples collected from supervised field sprayed with 100, 200 and 300g a.i./ha.

Days after treatment	Recovery of residues (mg kg^{-1})					
	Sample extracted with ethyl acetate residues%			Sample extracted with acetonitrile residues%		
	dose (100 g a.i. ha^{-1})	dose (200 g a.i. ha^{-1})	dose (300 a.i. ha^{-1})	dose (100 a.i. ha^{-1})	dose (200 a.i. ha^{-1})	dose (300 a.i. ha^{-1})
0 (1hr)	0.374	0.682	0.862	0.378	0.685	0.862
1	0.242	0.421	0.509	0.244	0.424	0.509
3	0.140	0.261	0.280	0.143	0.269	0.284
5	0.062	0.121	0.144	0.062	0.125	0.146
7	0.051	0.062	0.061	0.054	0.064	0.065
9	0.020	0.025	0.032	0.024	0.025	0.032
11	0.008	0.017	0.025	0.008	0.018	0.025
13	0.004	0.009	0.015	0.004	0.010	0.016
15	BDL	0.002	0.008	BDL	0.002	0.008
17		BDL	0.003		BDL	0.003
19			BDL			BDL

Table: 9 Extraction of monocrotophos residue using different solvent system in okra samples collected from supervised field sprayed with 100, 200 and 300g a.i./ha.

Days after treatment	Recovery of residues (mg kg^{-1})					
	Sample extracted with ethyl acetate residues%			Sample extracted with acetonitrile residues%		
	dose (100 g a.i. ha^{-1})	dose (200 g a.i. ha^{-1})	dose (300 a.i. ha^{-1})	dose (100 a.i. ha^{-1})	dose (200 a.i. ha^{-1})	dose (300 a.i. ha^{-1})
0 (1hr)	0.389	0.695	0.898	0.389	0.698	0.898
1	0.254	0.434	0.529	0.256	0.435	0.529
3	0.159	0.271	0.302	0.159	0.270	0.302
5	0.084	0.131	0.152	0.089	0.131	0.156
7	0.049	0.065	0.0.72	0.049	0.066	0.072
9	0.035	0.042	0.054	0.039	0.045	0.056
11	0.019	0.037	0.045	0.019	0.039	0.045
13	0.009	0.018	0.023	0.009	0.018	0.023
15	BDL	0.007	0.012	BDL	0.007	0.012
17		BDL	0.004		BDL	0.004
19			BDL			BDL

BDL: Below Detection Limit

4. CONCLUSION

In this study it was evaluated the suitability of two solvents for GC-MS analysis of multiclass pesticide residues. MeCN offers advantages in extraction selectivity and compatibility with more diverse analytical techniques compared to EtAc. To conclude, MeCN is the most suitable solvent for extraction of a wide polarity range of pesticide residues from produce. After acidification, the stability of problematic pesticides in MeCN is acceptable, and MeCN can also serve as an adequate medium for the GC injection, therefore solvent exchange is necessarily not required prior to GC analysis

References

1. T. Cairns, J. Sherma (Editors), Emerging Strategies for Pesticide Analysis, CRC Press, Boca Raton, Florida, USA, 1992.

2. Lee, S.M., Papathakis, M.L., Feng, H.M., Hunter, G.F. & Carr. J.E. (1991) Multipesticide residue method for fruits and vegetables. Fresenius J. Anal. Chem., **339**, 376 – 383.

3**.** Steven J. Lehotay, Kyung Ae Son, Hyeyoung Kwon, Urairat Koesukwiwat, Wusheng Fu, Katerina Mastovskaa, Eunha Hoh, Natchanun Leepipatpiboon, Comparison of QuEChERS sample preparation methods for the analysis ofpesticide residues in fruits and vegetables, Journal of Chromatography A, 1217 (2010) 2548–2560.

4. Schenck FJ, Brown AN, Podhorniak LV, Parker A, Reliford M, Wong JW. A rapid multiresidue method for determination of pesticides in fruits and vegetables by using acetonitrile extraction/partitioning and solid-phase extraction column cleanup. J AOAC Int. 2008,91(2):422-38.

5. M. Anastassiades, S.J. Lehotay, D. Stajnbaher, F.J. Schenck, 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 (2003) 412-415.

6. W. Liao, T. Joe, W.G. Cusick, Minimization of solvent consumption in pesticide residue analysis J. AOAC Int. 74 (1991) 554.

7. R.S. Sheridan, J.R. Meola, Analysis of pesticide residues in fruits, vegetables, and milk by gas chromatography/tandem mass spectrometry.J. AOAC Int. 82 (1999) 982-990.

8. J. Fillion, F. Sauve, J. Selwyn, Multiresidue method for the determination of residues of 251 pesticides in fruits and vegetables by gas chromatography/mass spectrometry and liquid chromatography with fluorescence detection.J. AOAC Int. 83 (2000) 698.

9. Lehotay S.J.(2007). Determination of pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate: collaborative study, J AOAC Int.90(2):485-520.

10. EN 12393-2: Non-Fatty Foodstuffs-Multiresidue Methods for the Gas Chromatographic Determination of Pesticide Residues. Part 2.Methods for Extraction and Clean-Up, European Committee for Standardization, Brussels, 1999.

11. A. Andersson, H. Pahsleden, Fresenius Comparison of the efficiency of different GLC multi-residue methods on crops containing pesticide residues J. Anal. Chem. 339 (1991)365-367.

12. Amadeo R., Fernandez Alba, Ana Tejedor and Ana Aguera: Determination of Imidacloprid and Benzimidazole Residues in Fruits and Vegetables by Liquid Chromatography–Mass Spectrometry after Ethyl Acetate Multiresidue Extraction .J. AOAC Int. 83, (3), 2000,748-755.

13. D.M. Holstege, D.L. Scharberg, E.R. Tor, L.C. Hart, F.D. Galey, A rapid multiresidue screen for organophosphorus, organochlorine, and N-methyl carbamate insecticides in plant and animal tissues J.AOAC Int. 77(1994)1263-74.

14. Perihan Aysal, Arpad Ambrus, Stewen J. Lehotay and Andrew Cannavan(2007). Validation of an efficient method for the determination of pesticide residues in fruits and vegetables using ethyl acetate for extraction, Journal of Environmental Science and Health Part B,42. 481-490.

15.Hans G. J. Mol & Astrid Rooseboom & Ruud van Dam &Marleen Roding & Karin Arondeus & Suryati Sunarto Modification and re-validation of the ethyl acetate-based multi-residue method for pesticides in produce Anal Bioanal Chem (2007) 389:1715–1754.

16.Berrada H, Fernández M, Ruiz MJ, Molto JC, Mañes J. Exposure assessment of fruits contaminated with pesticide residues from Valencia, 2001- 03.Food Addit Contam. 2006 Jul;23(7):674-82.

17. Stajnbaher, D. and Zupancic-Kral, L. (2008). Optimisation of programmable temperature vaporizer-based large volume injection for determination of pesticide residues in fruits and vegetables using gas chromatography–mass spectrometry. J. Chromatogr.A. 1109. 316-326.

18. Katerina Mastovska, Steven J. Lehotay Evaluation of common organic solvents for gas chromatographic analysis and stability of multiclass pesticide residues Journal of Chromatography A, 1040 (2004) 259–272.

19. E.W. Washburn (Ed.), International Critical Tables of Numerical Data, Physics, Chemistry and Technology, first electronic ed., Knovel, Norwich, New York, 2003.

20. S.J. Lehotay, A.R. Lightfield, J.A. Harman-Fetcho, D.A. Donoghue,J. Agric. Food Chem. 49 (2001) 4589. V. Leoni, J. Assoc. Off. Anal. Chem. 75 (1992) 511.