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ORIENTAL JOURNAL OF CHEMISTRY
An International Open Access, Peer Reviewed Research Journal

www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2023, Vol. 39, No.(5):
Pg. 1145-1155

Development of A Multi Residue Method for the Quantification of 45 Pesticides using GC-MS/MS and Study of Peeling Effect on Pesticide Residues in Citrus Fruits

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http://dx.doi.org/10.13005/ojc/390507

(Received: June 30, 2023; Accepted: October 16, 2023)

ABSTRACT

An analytical method was developed and validated for the determination of 45 multi-class pesticide residues in citrus fruit samples collected from and around Pulivendula, India, using GC-MS/MS (Gas chromatography with tandem mass spectrometry) followed by the QuEChERS extraction method. The linear regression coefficients (R-square) of the methods range from 0.998 to 0.999, and the Limit of Detection (LOD) and Limit of Quantification (LOQ) are 1.56 to 25.23 ng/mL and 4.72 to 76.47 ng/mL, respectively. Recoveries of all spiked pesticides range from 82.6 to 117.6%, with a RSD (Relative Standard Deviation) less than 11.2%. The results show that 42 out of 45 pesticides were detected in whole citrus fruit pulp (with peel) samples. Fenthion, bifenthrin, and fenvalerate were not detected. In the collected citrus samples, phorate (21.71 μg/kg), and ethion (51.47 μg/kg) insecticides are present above the Maximum Residue Level (MRL), but cypermethrin (25.89 μg/kg) was detected below the MRL. 13 out of 45 pesticides were detected in edible parts of citrus fruit (without peel) samples, with ethion having the highest residue. All pesticides were within the MRL limits prescribed by the European Union (EU) and Codex regulations for MRL in citrus fruits, and peeling was found to be one of the best ways to get rid of pesticide residues.

Keywords: QuEChERS extraction, GC-MS/MS, 45 multi-class pesticides, pesticide residues and citrus fruits.

INTRODUCTION

The Rutaceae family, which includes citrus fruits, is the most well-known and commonly cultivated fruit family in the world, with an annual production of 158 million metric tonnes[1]. China is the world's leading producer of citrus, with 44 million metric tonnes (approximately 28% of total production). With 19.7 million metric tonnes, Brazil comes in second, followed by 14 million metric tonnes for India. Citrus fruits are India's third most produced fruit, behind mango and banana. 26

states in total, about 1.04 million acres, are used for agriculture[2].

In order to safeguard consumer health and promote ethical practises in the food trade, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) established the Codex Alimentarius Commission (CAC) in 1963. The CAC develops standardised international food standards, guidelines, and codes of practise. The Commission also encourages worldwide governmental and non-governmental groups to coordinate all of their efforts in the area of food standards[3]. Regulations from the European Union (EU) outline the maximum residual limit (MRL) for pesticides in products with plant origins[4]. The second important organisation is the Food Safety and Standards Authority of India (FSSAI). The Ministry of Health and Family Welfare formed the FSSAI in accordance with the Food Safety and Standards act of 2006, and it is responsible for recommending tolerance levels for certain pesticides in food commodities[5].

Gas chromatography-tandem mass spectrometry (GC-MS/MS), which has the ability to separate coeluting compounds based on compound-specific target-oriented multiple reaction monitoring (MRM) transitions, appears to be a potent technique for overcoming these issues[6,7]. The extraction and detection methods for estimating the presence of pesticide residues in fruits and vegetables are summarized in Table 1.

In a recent study, the active ingredients in the veggie and fruit samples were chlorpyrifos, malathion, dieldrin, boscalid, triticonazol, difenocpnazol, acetamiprid, azoxystrobin, tebuconazole, trifloxystrobin, pirimicarb, and dodine. But in six of the samples, the amount of active chemicals was above the maximum residue levels (MRL). The different methods used in the study showed that peeling was the best way to get rid of pesticide residue. The washing process also showed that it got rid of some poisons but didn't get rid of all of them[8].

Table 1: An overview of extraction methods, detection techniques, recovery (%), LOD and LOQ of pesticides in fruits and vegetable

S. No	Method of Extraction	Method of Separation /Detection	No of target pesticides	Recovery(%)	Type of Fruit & Vegetables	LOD mg/kg	LOQ mg/kg	Reference
1	Liquid-liquid Extraction (LLE)	UHPLC-QqQ /TOF/QTOF	11-Multi-class pesticides	70-100	Orange, tomato, grape fruit, cucumber and pepper, banana, strawberry,	0.01	None	[9]
2	Liquid-liquid microextraction (LLME)	GC-MS/MS	Pyrethroid insecticides	73-92	Fruits and fruit juices	0.006-0.038	0.023-0.121	[10]
3	QuEChERS	GC-MS-TOF	55-multi-class pesticides	70-120	Apple, tomatoes, carrot, oranges and olives	None	0.01 -0.5	[11]
4	QuEChERS	GC-MS-MSD (NCI)	25- multi-class pesticides	70-110	Apple, oranges, strawberry, plum	None	None	[12]
5	QuEChERS	GC-MS/MS (QqQ)	140- multi-class pesticides	70-110	Cucumber and orange	0.006	0.008	[13]
6	QuEChERS	GC-MS/MS	THI, IMD	70-120	Orange fruits	None	None	[14]
7	DMD & EBD	LC-MS/MS, LC-MS	Dithiocarbamate	97-101	Cucumbers, Apples, pears, grapes, cherry, tomatoes, tamarillos, papaya and broccoli	0.001	0.005	[15]
8	QuEChERS	UHPLC-MS/MS	88-pesticides	None	Citrus Fruits juice	0.0034	None	[16]

When eating citrus fruits, it's normal to peel them first. Lemons, on the other hand, are often used without being peeled to make spices[17]. The goal of this study was to develop and validate a multi-residue method to examine the 45 pesticide residues in citrus fruits (sweet lime and lemon) collected from three different forming lands at Ankalamma Guduru, Duddekunta, and Kadapanagaya Palli in and around Pulivendula using GC-MS/MS. Further, the effect of home cleaning methods on pesticide residue in citrus fruits (sweet lime and lemon) was also analyzed.

MATERIALS AND METHODS

Chemicals and Reagents

The 45 pesticides specified in Table 2, that have technical-grade standards with purity levels of ≥96% were bought from M/S Sigma Aldrich in Bengaluru, Karnataka, India, for use in this study. Local suppliers also provided QuEChERS extraction solvents such as ethyl acetate ($C_4H_8O_2$), sodium acetate, $MgSO_4$, and PSA.

Selection of Sampling area

As per the YSR Kadapa district survey report for the year 2021–22 given by the Andhra Pradesh Space Applications Centre (APSAC) ITE and C Department, Govt. of Andhra Pradesh[18], the total land used for cultivating orange and batavia is nearly 69991 Acres with 438928 MT of production. Among those, 66% of total production came from Pulivendula Tehsil alone, with citrus cultivation land of approximately 45000 Acres. So, the sampling areas are selected in and around the Pulivendula, namely Ankalamma Guduru (14°34'52.0"N 78°10'55.7"E), Duddekunta (14°37'14.5"N 78°12'15.6"E), and Kadapanagaya Palli (14°36'43.1"N 78°11'41.7"E).

Table 2: List of Retention times (R_t), MRM transitions (m/Z), reference ion, collision energy (CE), R-square, Regression Equation, LOD, and LOQ

S.No	Pesticide Name	R_t (min)	m/Z	Reference ion	CE (eV)	R-square	Regression Equation	LOD (ppb)	LOQ (ppb)
1	Dichlorvos	6.846	185.0->93.0	185.0->109.0, 220.0->185.0	10	0.99983	y=13535.801057* x-30738.801642	2.62	7.93
2	HCH Alpha	10.903	181.0->145.0	216.9->145.0, 216.9->181.0	10	0.99992	y=23205.073825* x-40391.556774	2.31	7.01
3	Phorate	10.782	260.0->75.0	260.0->231.0, 121.0->120.2	10	0.98156	y=17.364207* x-56.245683	3.73	10.93
4	Hexachloro Benzene	11.062	249.0->213.9	284.0-> 248.9, 284.0->213.9	20	0.99999	y=22540.757782* x-20725.619933	1.56	4.72
5	PCNB	11.062	249.0->214.0	214.0->179.0, 237.0->143.0	15	0.99999	y=22540.757782* x-20725.619933	1.56	4.72
6	Dimethoate	11.144	125.0->47.0	125.0->79.0, 229.0->87.0	15	0.99924	y=13077.721688* x-20886.312248	6.53	19.79
7	HCH beta	11.535	180.8->145.0	183.0->147.0	15	0.99971	y=24554.821803* x-54489.655006	3.54	10.73
8	HCH Gamma	11.995	180.8->145.0	183.0->147.0	5	0.99911	y=19281.415473* x-50340.829472	7.30	22.12
9	Diazinon	11.805	137.1->84.0	179.0->137.0, 199.0->93.0	10	0.99944	y=9921.136162* x-15179.248096	8.45	25.59
10	HCH Delta	12.001	219.0->147.0	217.0->145.0	15	0.99775	y=2389.343405* x-7861.705503	17.78	53.87
11	Phosphomidon	12.566	264.0->127.0	127.0->95.0, 192.9->127.0	15	0.99628	y=3193.648941* x-13219.592271	16.86	51.08
12	Methyl Parathion	13.270	125.0->47.0	263.0->246.0, 263.0->109.0	10	0.99911	y=17820.228893 * x-38530.637212	8.13	24.62
13	Chlorpyrifos Methyl	12.748	285.9->92.9	286.0->241.0, 286.0->208.0	20	0.99975	y=12281.428410* x-26070.029620	3.44	10.41
14	HeptaChlor	12.918	271.7->236.9	270.0->235.0, 272.0->237.0	15	0.99969	y=35702.003593* x-64128.740508	4.74	14.37
15	Malathion	13.454	126.9->99.0	157.8->125.0, 173.0->127.0	10	0.99800	y=37846.919104* x-121311.361026	12.78	38.72
16	Paraxon Ethyl	13.267	247.0->109.1	148.9->119.0, 220.0->174.1	20	0.99717	y=1951.810374* x-4093.913450	21.61	65.47

17	Fenitrothion	13.265	277.0->109.0	277.0->260.0	15	0.99403	y=4307.870163*x-17097.284311	25.23	76.47
18	Pirimiphos Methyl	13.285	290.0->125.0	290.0->233.0, 305.0->276.0	2	0.99866	y=4315.877055*x-6758.433389	10.00	30.30
19	fenthion	13.633	278.0->109.0	278.0->245.1, 278.0->169.1	15	0.99793	y=202.940087*x+1336.475654	13.81	41.85
20	Aldrin	13.592	262.9->192.9	262.9->190.9, 254.9->220.0	35	0.99933	y=17824.013709*x-15928.701717	11.23	34.02
21	Parathion	13.689	291.0->109.0	291.0->263.0, 139.0->109.0	15	0.99536	y=3598.490725*x-16995.502167	17.38	52.67
22	chloropyrifos	13.672	198.9->171.0	196.9->169.0, 313.8->257.8	15	0.99965	y=21414.846187*x-33668.299983	3.69	11.17
23	Dicofol	13.735	250.0->139.0	251.0->139.0, 253.0->141.0	15	0.99935	y=12429.267744*x-11780.915771	7.07	21.41
24	HeptaChlor Epoxide	14.349	353.0->262.9	353.0->282.0, 353.0->217.1	10	0.99961	y=3438.057811*x-1718.309628	5.88	17.82
25	Alpha Endosulfon	15.035	241.0->206.0	194.9->125.0, 194.9->160.0	10	0.99964	y=3082.068273*x-3484.245645	3.90	11.82
26	Cis chlordane	15.080	373.0->266.0	373.0->264.0, 266.0->196.0	20	0.99961	y=8207.643701*x-9442.522499	6.41	19.42
27	TransChlordane	15.080	373.0->266.0	373.0->264.0	20	0.99961	y=8207.817818*x-9435.897798	6.39	19.35
28	2,4 DDE	15.447	246.0->176.1	318.0->246.1	30	0.99953	y=108929.164403*x-66759.369967	9.19	27.86
29	DDE	15.447	246.0->176.1	318.0->248.1, 318.0->246.1	30	0.99953	y=108929.164403*x-66759.369967	9.19	27.86
30	Beta Endosulfon	15.932	241.0->206.0	195.0->125.0, 195.0->160.0	10	0.99906	y=548.116580*x-272.513443	9.93	30.08
31	Dieldrin	15.931	262.9->193.0	262.9->191.0, 277.0->206.0	35	0.99930	y=9198.817347*x-2845.932902	10.99	33.30
32	endrin	15.931	262.8->193.0	244.8->210.0, 281.0->245.0	35	0.99930	y=9198.817347*x-2845.932902	10.99	33.30
33	DDD	16.232	235.0->165.1	235.0->200.1, 235.0->199.1,	35	0.99983	y=159025.732511*x-160552.398724	4.89	14.83
34	Endrin Aldehyde	16.451	281.0->245.0	249.9->214.9, 344.9->244.9	10	0.99958	y=1857.919198*x-4192.028492	8.12	24.61
35	4,4 DDT	16.232	235.0->165.0	235.0->199.0	20	0.99983	y=159026.158530*x-160518.303409	4.96	15.03
36	Ethion	16.309	231.0->175.0	231.0->203.0, 231.0->129.0	10	0.99876	y=28780.040853*x-77709.304562	10.10	30.62
37	Endosulfan Sulfate	16.884	272.0->236.9	272.0->234.9, 387.0->206.0	10	0.99881	y=6439.247511*x-27176.009390	8.55	25.90
38	Endrin Ketone	17.743	317.0->281.0	281.0->245.0, 317.0->245.0	5	0.99784	y=297.168231*x-1171.496159	11.60	35.16
39	Methoxychlor	17.950	227.1->169.1	227.1->212.2, 227.1->141.1	20	0.99932	y=19226.409644*x-50328.270146	9.81	29.72
40	Bifenthrin	17.856	181.2->165.2	165.0->164.1, 166.2->165.2	25	0.99923	y=148026.797780*x+72570.672075	11.64	35.27
41	Phosalone	18.507	367.0->182.0	182.0->102.1, 182.0->75.1	10	0.99658	y=5417.104659*x-20318.177402	16.16	48.97
42	Cypermethrin	19.853	163.0->127.0	165.1->127.0, 181.0->151.0,	10	0.99960	y=26881.983252*x-45465.169362	6.03	18.27
43	Permethrin	19.853	163.0->127.0	183.1->165.1, 183.1->153.1,	10	0.99960	y=26881.983252*x-45465.169362	6.03	18.27
44	Fenvalerate	21.103	167.0->125.1	225.0->119.1	5	0.99736	y=4125.749123*x+21323.916736	15.91	48.20
45	Deltamethrin	24.373	253.0->174.1	252.9->93.0, 253.0->172.0	10	0.99866	y=788.534919*x-2759.529094	9.64	29.21

R_t-Retention time in minutes, R-square- Coefficient of Determination, m/Z-MRM transitions, LOD-Limit of Detection in ng/mL CE-Collision Energy in eV, LOQ-Limit of Quantification in ng/mL.

Sample Collection and Pre-processing

The citrus fruits, around 80 nos., were collected from three different forming lands mentioned above and subsamples were mixed to make a single sample from each land. Three different types of citrus fruits, namely FS1 (ripe sweet lime fruit, 7 months old), FS2 (raw sweet lime fruit, 2 months old), and FS3 (ripe lemon, 7 months old), were collected, cleaned thoroughly with water, and sent to the laboratory for further analysis. Whole citrus fruits and edible parts of citrus fruits were extracted from the fruit samples separately. Further, the samples are blended and homogenized and kept at 2-4°C for further analysis.

Instrumentation

The 45 multi-class pesticides are extracted using the chosen GC-MS/MS instrument optimised for citrus fruit samples. The mass spectrometer, a Shimadzu GCMSTQ8040NX model from Japan is utilized in the present study. It is also connected to a GC-2010 Plus that has an AOC-20i autosampler and a 20S autoinjector fitted. 15 metres, 250 m ID, 0.25 m length SX-Rxi-5 Sil MS capillary column was employed. As the carrier gas, helium was used at a constant level of pressure that was changed every day. The gas chromatograph's intake was lined with glass wool inside of a splitless GC glass liner from Shimadzu, Japan. The injection port and transfer line for the mass spectrometer were both 220°C with a 1 mL/min column flow and a 1.1 mL/min purge flow, and the electron energy of the EI positive was 70 eV. The temperature programme used to operate the GC oven was as follows: the starting point of 50°C held for 0.5 min, scaled at 120°C/min to 60°C held for 1.5 min, afterwards an increase of 25°C/min to 170°C held for 1 min, followed by a ramp of 10°C/min to 280°C maintained for 7 minute. The contact area was kept at 250°C, the source of ions was set at 230°C, and the M/Z (scan) range was within 50 and 500 for a total run time of 25.48 minutes.

Preparation of Standard Pesticide Solutions

Weigh 5 mg of an individual clean standard into a 10 mL individual volumetric flask, make up to the mark with ethyl acetate, and store at 2–8°C. The right amount of each standard stock solution, derived from its concentration, is transferred into a 10 mL volumetric flask and then diluted with ethyl acetate to create working standard solutions (1 mg/L). Label the solutions, and then keep them between 2 and 8°C. Prepare the calibration curve standards using ethyl acetate and label them with concentrations ranging from 10 ng/mL to 200 ng/mL. By mixing appropriate amounts of each pesticide to make spike solutions at 10, 50, and 100 ng/mL.

Multiclass Residue Extraction and Clean-up

Samples of citrus (sweet lime and lemon) were carefully mixed in ambient light. After further high-speed homogenization of the blended sample (200 g), 10 g of homogenous material were weighed into a 50 mL centrifuge tube. Twenty minutes later, add 10 mL of ethyl acetate and vortex. Add the 1.5 g sodium acetate and 6 g $MgSO_4$ from the QuEChERS extraction kit. For 30 seconds, shake. The sample should be centrifuged at 6000 RPMs for 5 minutes at 20°C. Take 1 mL of supernatant into a 2 mL dispersion tube (5982-0028 CH). Vortex for 1 min and centrifuge the sample at 9000 rpm for 10 min at 20°C. Collect Supernant that filtered through 0.2 µm polytetrafluoroethylene membrane filter transfer in to auto sampler vial for analysis by GC-MS/MS technique.

Method development

Throughout the development of the method, linearity, LOD, LOQ, accuracy, and precision were all validated. The apparatus must be calibrated in order to conduct an accurate analysis. The ability of an analytical procedure to produce results from tests that are directly proportional to the concentration of an analyte in the sample is referred to as linearity. Calibration curve standards of five concentrations, namely 10, 25, 50, 100, and 200 ppb, were injected into GC-MS/MS. The diagram of responses as a function of analyte concentration is evaluated using a typical regression analysis with a minimum of five linear concentrations. With a signal-to-noise (S/N) ratio of 3 and 10, respectively, the least concentration was used to calculate the LOD and LOQ for each pesticide.

Method validation

The spiking pesticide standards containing all 45 multiclass pesticides at three concentrations of 10, 50, and 100 ppb were used to study recoveries and validate the developed method. The accuracy and precision of the approach were evaluated in recovery trials with three spike levels in three replicates. The acceptable pesticide recoveries have a Relative Standard Deviation (RSD n=3) <11.2% and fall between 82.6 and 117.6%. Pesticide retention times in sample extracts were matched with a tolerance of 0.1 min by the average retention periods of the calibration standards recorded in the same analytical process[19].

RESULT AND DISCUSSIONS

Method development

A modified QuEChERS extraction method with d-SPE clean-up followed by GC-MS/MS was developed for the detection of 45 multi-class pesticides in citrus fruits. Multi-residue method to examine the 45 pesticide residues in citrus fruits (sweet lime and lemon) collected from three different forming lands at Ankalamma Guduru, Duddekunta, and Kadapanagaya Palli in and around Pulivendula. Further, the effect of home cleaning methods on pesticide residue in citrus fruits (sweet lime and lemon) was also analyzed.

The linearity of each pesticide was tested at five different concentrations of calibration curve standards: 10, 25, 50, 100, and 200 ng/mL, or ppb. All pesticides' mass spectral responses were linear in the examined concentration range, with determinant coefficients >0.981. Table 3 provides a summary of the calibration data for the pesticides under investigation.

By introducing 1 µL of a 100 ng/mL mixed pesticide standard mixture into the GC-MS/MS, the MRM transitions and associated acquisition settings were tuned for the highest response of the fragmented ions under EI positive mode. The most sensitive product ions were then determined by testing various collision energies after using helium gas to initiate dissociation. Various pesticide residues in actual samples were quantified using the optimized parent m/z and product ion transitions with CE. For the analytical approach used to detect multiclass pesticides at their lowest levels in citrus fruits, the developed GC- MRM mode offers high sensitivity and selectivity criteria.

Figure 1 shows the GC-MS/MS total ion chromatogram (TIC) of the total pesticide standard mixture at 100 ng/mL concentrations, which was used to correctly identify all of the pesticides. Table 3 includes a list of pesticides along with details on their retention times R(t), MRM Transition (m/Z), reference ion, collision energy (eV), and retention times. By identifying the target and qualifier ions and calculating the qualifier-to-target ratio, the presences of pesticides were confirmed. Also, the set of regression equations, R-square values, LOD (limit of detection), and LOQ (limit of quantification) were reported. The coefficients of determination of methods range from 0.981 to 0.999. Comparatively, the limit of detection 1.56 to 25.23 ng/mL and limit of quantitation are 4.72 to 76.47 ng/mL compared to other studies published on the same matrices.

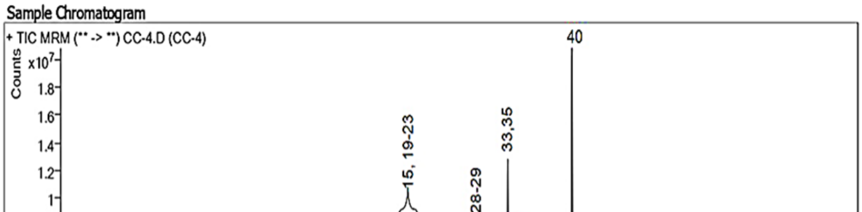

Fig. 1. GC/MS-MS Total Ion Chromatogram (TIC) of the pesticide standard mixture (100 ng/mL). Peaks: ES: Ethyl Acetate, 1: Dichlorvos, 2: HCH Alpha, 3: Phorate, 4: Hexachloro Benzene, 5: PCNB, 6: Dimethoate, 7: HCH beta, 8: Gamma-HCH, 9: Diazinon, 10: HCH delta, 11: Phosphomidon, 12: Methyl parathion, 13: Chlorpyrifos mehtyl, 14: Heptachlor, 15: Malathion, 16: ParaxonEthyl, 17: Fenitrothion, 18: Pirimiphos Methyl, 19: Fenthion, 20: Aldrin, 21: Parathion, 22: Chlorpyrifos, 23: Dicofol, 24: Hepta Chlor Epoxide, 25: Alpha Endosulfon, 26: Cis chlordane, 27: Trans Chlordane, 28: 2,4 DDE, 29: DDE, 30: Beta Endosulfon, 31: Dieldrin, 32: Endrin, 33: DDD, 34: Endrin Aldehyde, 35: 4,4 DDT, 36: Ethion, 37: Endosulfan Sulphate, 38: Endrin Ketone, 39: Methoxychlor, 40: Bifenthrin, 41: Phosalone, 42: Cypermethrin, 43: Permethrin, 44: Fenvalerate, and 45: Deltamethrin

Method validation

The results of recoveries at three different fortification levels (10, 50, and 100 ng/mL) and their relative standards (n=3) are plotted in Fig. 2. This method has a high degree of accuracy (between 82.6% and 117.6% of recovery), reproducibility (1.1 to 11.2 of RSD), and robustness, making it suitable for large-scale monitoring of citrus fruits gathered from farmer's fields. The obtained R-square, LOD, LOQ, and recoveries of all pesticides are good when compared to other findings in the citrus fruit matrix[4,14,16,20-25].

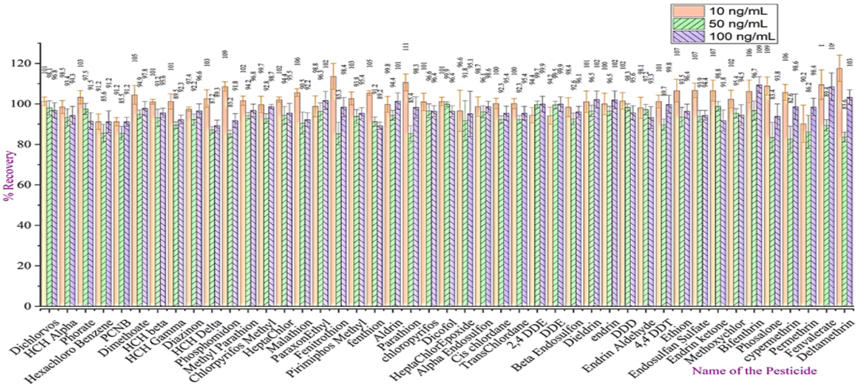

Fig. 2. Average recoveries (%) and relative standard deviations (%) of pesticides obtained by GC-MS/MS analysis of citrus samples at 3 spiking levels (n=3)

Application of the Developed Technique

The citrus fruits, around 80 nos., were collected from three different forming lands located at Ankalamma Guduru, Duddekunta, and Kadapanagaya Palli and thoroughly mixed to make a single sample. Three different types of citrus fruits, namely FS1 (ripe sweet lime fruit, 7 months old), FS2 (raw sweet lime fruit, 2 months old), and FS3 (ripe lemon, 7 months old), were collected, cleaned thoroughly with water, and sent to the laboratory for further analysis. Whole citrus fruit (with peel) and edible parts of citrus fruits (without peel) were taken from the fruit samples separately. Further, the samples are grinded and kept at 2-4°C for further analysis. The developed GC-MS/MS technique was used to extract multi-class pesticide residues from the aforementioned samples, and the results are tabulated in Table 3.

Table 3: Pesticide residues detected on three citrus samples with peel and without peel

S. No	Pesticide	Whole Fruit(with peel) µg/kg			Edible part(without peel) µg/kg			MRL1	MRL2
		FS1*	FS2*	FS3*	FS1*	FS2*	FS3*		
1	Dichlorvos	2.278	2.274	2.278	ND	1.645	ND	10	NA
2	HCH Alpha	1.747	1.750	1.768	0.672	0.684	0.698	10	NA
3	Phorate	13.587	21.66	21.71	2.225	2.714	3.654	10	NA
4	Hexachloro Benzene	0.961	0.959	0.946	ND	ND	ND	10	NA
5	PCNB	0.961	0.959	0.946	ND	ND	ND	NA	NA
6	Dimethoate	1.629	1.750	1.701	ND	ND	ND	10	5000
7	HCH beta	2.229	2.276	2.228	0.547	0.644	0.632	10	NA
8	HCH Gamma	2.624	2.625	2.624	ND	ND	ND	10	NA
9	Diazinon	1.550	1.586	1.587	ND	ND	ND	10	NA
10	HCH Delta	3.390	4.896	3.805	1.332	1.430	1.201	NA	NA
11	Phosphomidon	4.235	4.246	4.247	1.323	1.118	1.164	NA	NA
12	Methyl Parathion	2.170	2.173	2.228	0.514	ND	0.175	NA	NA
13	Chlorpyrifos Methyl	2.132	2.148	2.151	1.252	1.125	1.712	10	2000
14	HeptaChlor	1.804	1.803	1.804	ND	ND	ND	10	10
15	Malathion	3.229	3.627	5.002	2.277	2.438	1.878	20	7000
16	ParaxonEthy	2.171	2.695	2.597	ND	ND	ND	NA	NA
17	Fenitrothion	4.026	4.513	4.763	ND	ND	ND	10	NA
18	Pirimiphos Methyl	1.581	1.598	1.592	ND	ND	ND	10	NA
19	Fenthion	ND	ND	ND	ND	ND	ND	10	2000

20	Aldrin	0.922	1.192	1.119	ND	ND	ND	10	50
21	Parathion	4.741	5.240	5.327	1.457	1.222	1.722	50	NA
22	Chlorpyrifos	1.584	1.629	1.627	0.996	0.845	0.758	10	1000
23	Dicofol	0.973	0.969	1.028	ND	ND	ND	20	NA
24	HeptaChlorEpoxide	0.604	0.555	0.581	ND	ND	ND	NA	NA
25	Alpha Endosulfon	1.174	1.206	1.245	ND	ND	ND	50	NA
26	Cis chlordane	1.166	1.181	1.171	ND	ND	ND	10	NA
27	TransChlordane	1.165	1.180	1.205	ND	ND	ND	NA	NA
28	2,4 DDE	0.615	0.618	0.624	ND	ND	ND	1000	1000
29	DDE	0.615	0.618	0.624	ND	ND	ND	NA	NA
30	Beta Endosulfon	0.742	0.803	0.836	ND	ND	ND	50	NA
31	Dieldrin	1.008	0.713	0.650	ND	ND	ND	10	50
32	Endrin	1.008	0.713	0.650	ND	ND	ND	10	NA
33	DDD	1.037	1.108	1.091	ND	ND	ND	NA	NA
34	Endrin Aldehyde	2.285	2.402	2.392	ND	ND	ND	NA	NA
35	4,4 DDT	1.015	1.035	1.091	0.746	0.544	0.788	50	NA
36	Ethion	40.51	41.55	51.47	4.173	4.755	3.932	10	NA
37	Endosulfan Sulfate	4.237	4.258	4.237	ND	ND	ND	NA	NA
38	Endrin Ketone	4.027	4.179	4.044	ND	ND	ND	NA	NA
39	Methoxychlor	2.620	2.690	2.628	ND	ND	ND	10	NA
40	Bifenthrin	ND	ND	ND	ND	ND	ND	50	50
41	Phosalone	3.759	3.759	3.759	ND	ND	ND	10	NA
42	Cypermethrin	25.89	24.14	24.15	3.145	3.915	3.005	2000	200
43	Permethrin	1.719	1.998	1.718	0.555	0.855	0.854	50	500
44	Fenvalerate	ND	ND	ND	ND	ND	ND	20	NA
45	Deltamethrin	3.590	3.677	3.691	ND	ND	ND	40	20

1Maximum Residue Limit - EU Regulations maximum residue limit in citrus fruits in ng/mL

2Maximum Residue Limit - Codex preferred maximum residue limit in citrus fruits and its products in µg/kg

FS1 (ripe sweet lime fruit, 7 months old),

FS2 (raw sweet lime fruit, 2 months old), and

FS3 (ripe lemon, 7 months old)

NA-Not Available

Whole Citrus Fruit (with peel)

The results show that 42 out of 45 pesticides were detected in whole citrus fruit pulp (with peel) samples. Fenthion, bifenthrin, and fenvalerate were not detected. In the collected citrus samples, phorate (21.71 µg/kg highest in ripe lemon), and ethion (51.47 µg/kg highest in ripe lemon), insecticides are present above the maximum residue limit (MRL) level, but cypermethrin (25.89 µg/kg highest in ripe sweet lime) was detected below the MRL, as shown in Fig. 3. Numerical data for the graph is reported in Table 3. Ethion and cypermethrin are the most commonly used pesticides in citrus orchards. During the study period, both pesticides were sprayed twice each. Surprisingly, phorate was found in whole citrus fruit, but due to its extreme toxicity to mammals, fish, and birds, it was banned in India in 2021. Except for phorate and ethion remaining, all pesticide residues are below the MRL prescribed by the EU, and Codex regulations for MRL in citrus fruits are reported in Table 3.

Edible Parts of Citrus Fruit (without peel)

The results show that 13 out of 45 pesticides were detected in edible parts of citrus fruit (without peel) samples. HCH Alpha, Phorate, HCH Beta, Phosphomidon, Chlorpyrifos Methyl, Malathion, Parathion, Chlorpyrifos, 4.4 DDT, Ethion, Cypermethrin, and Permethrin are detected in edible parts of citrus fruit (without peel) samples. Among all pesticides, ethion (4.755 µg/kg highest in raw sweet lime) has the highest residue in citrus fruit, as shown in Fig. 4. Numerical data for the graph are reported in Table 4. When it comes to the MRL, all the detected pesticides are within the MRL limits prescribed by the EU and Codex regulations for MRL in citrus fruits.

When comparing the results from Fig. 3 and 4, the residues of pesticides in edible parts of the citrus fruits were observed at a very low level when compared with the whole fruit (with peel). So once again, the study showed that peeling was one of the best ways to get rid of pesticide residues (around 90% reduction in pesticide residues) from the fruit sample.

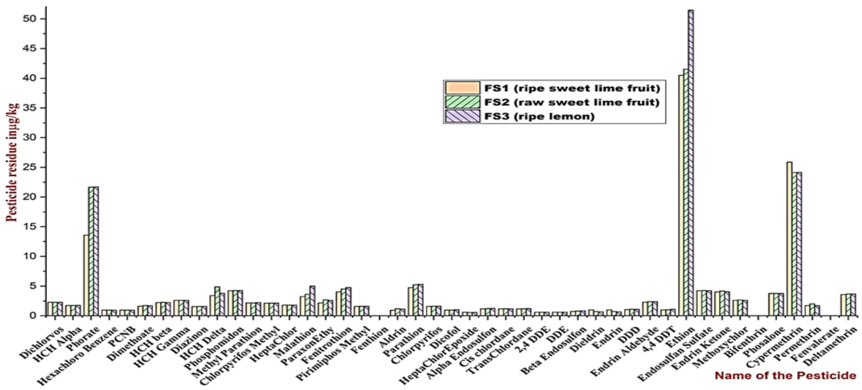

Fig. 3. Pesticide residue in whole fruit sample with peel (µg/kg)

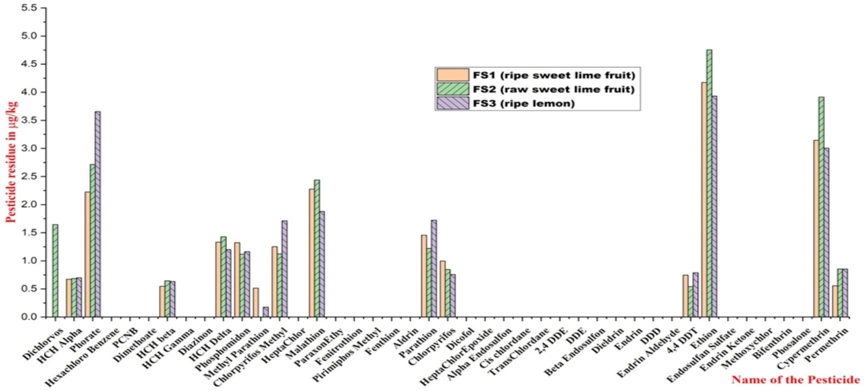

Fig. 4. Pesticide residue in whole fruit sample without peel (µg/kg)

CONCLUSION

In conclusion, the analytical method for simultaneous determination of 45 multi-class pesticide residues in three citrus fruit samples was successfully developed. The proposed optimised method is appropriate for rapidly (less than 25 min) screening citrus fruits for 45 diverse chemical pesticides. The linear regression coefficients of the methods range from 0.981 to 0.999. Comparatively, the limits of detection and quantitation are very low 1.56 to 25.23 ng/mL and 4.72 to 76.47 ng/mL compared to other studies published on the same matrices. This method has a high degree of accuracy (between 82.6% and 117.6%) with RSD 1.1-11.2% reproducibility, and robustness, making it suitable for large-scale monitoring of citrus fruits gathered from farmer's fields.

Further, the developed method was used to analyse the pesticides residues in citrus fruit samples. The results show that 42 out of 45 pesticides were detected in whole citrus fruit pulp (with peel) samples. Fenthion, bifenthrin, and fenvalerate were not detected. In the collected citrus samples, phorate (21.71 µg/kg highest in ripe lemon), and ethion (51.47 µg/kg highest in ripe lemon), insecticides are present above the maximum residue limit (MRL) level, but cypermethrin (25.89 µg/kg highest in ripe sweet lime) was detected below the MRL. Ethion and cypermethrin are the most commonly used pesticides in citrus orchards. During the study period, both pesticides were sprayed twice each. Surprisingly, phorate was found in whole citrus fruit pulp, but due to its extreme toxicity to mammals, fish, and birds, it was banned in India in 2021. Also, it is noticed that 13 out of 45 pesticides were detected in edible parts of citrus fruit (without peel) samples. HCH Alpha, Phorate, HCH Beta, Phosphomidon, Chlorpyrifos Methyl, Malathion, Parathion, Chlorpyrifos, 4.4 DDT, Ethion, Cypermethrin, and Permethrin are detected in edible parts of citrus fruit (without peel) samples. Among all pesticides, Ethion (4.755 µg/kg highest in raw sweet lime) has the highest residue in citrus fruits. When it comes to the MRL, all the detected pesticides in edible parts of the citrus fruits are within the MRL limits prescribed by the EU and Codex regulations for MRL in citrus fruits. So, the tested fruits are safe to eat after peeling.

ACKNOWLEDGMENT

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

Conflict of interest

The author declare that we have no conflict of interest.

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